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U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN No. 91.
A. D. MELVIN, CHIEF OF BUREAU.

FEEDING PRICKLY PEAR TO STOCK IN TEXAS.

BY

DAVID GRIFFITHS,

Assistant in Charge of Range and Cactus Investigations, Farm Management Investigations, Bureau of Plant Industry.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1906.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., July 28, 1906.

Sir: I have the honor to transmit herewith and to recommend for publication as a bulletin of this Bureau a manuscript entitled "Feeding Prickly Pear to Stock in Texas," by David Griffiths, assistant in charge of Range and Cactus Investigations, Farm Management Investigations, Bureau of Plant Industry. The accompanying letter from the Chief of the Bureau of Plant Industry explains why, although the work was done under the supervision of that Bureau, it seems desirable to publish the paper as a bulletin of the Bureau of Animal Industry.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

LETTER OF SUBMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Washington, D. C., July 5, 1906.

Sir: I have the pleasure to transmit the manuscript of a paper entitled "Feeding Prickly Pear to Stock in Texas," and recommend that it be published as a bulletin of the series of your Bureau. The investigations reported here are necessarily dual in character and deal with a subject partially within the province of investigation of both bureaus. It is a subject upon which we needed more information before proceeding further with investigations into the value of various species of cacti as farm and range crops. Inasmuch as the paper deals primarily with the animal side of the subject, I submit it to you for publication.

The paper was prepared by Dr. David Griffiths, assistant in charge of Range and Cactus Investigations, and has been submitted by the agriculturist in charge of Farm Management Investigations with a view to its publication. It is a continuation of Bulletin No. 74 of the Bureau of Plant Industry, which gives an account of the practice of stockmen in the use of cacti as forage plants, particularly in southwest Texas, where most of the cactus is fed.

The present paper gives an account of some experiments conducted by stockmen at the suggestion and under the direction of this Bureau. The results of these experiments are of unusual interest. The experiment with dairy cows was made in such a manner as to compare the cactus directly with sorghum hay. The two animals under experiment were fed at the beginning both cactus and sorghum. Their feed was then gradually changed to cactus. Afterwards one of them continued to receive cactus, while the other was changed gradually to sorghum hay. After a period of such feeding the feeds were gradually interchanged. During the whole of this test both cows were fed a mixed grain ration in addition to the roughage.

Generally speaking, the results indicate that cactus ad libitum produces a little better results in milk flow than sorghum hay ad libitum, both with sufficient grain, though the differences are small.

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The results indicate that 6 pounds of fresh cactus are equivalent in feeding value to 1 pound of dry sorghum hay. The test with beef cattle was undertaken to ascertain the cost of fattening cattle on cactus and cotton-seed meal, a common practice in the region where the cactus is mostly fed. The carload of steers used in the test made only moderate gains, averaging 1¾ pounds daily for each head during the fattening period. The very interesting result was obtained, however, that this gain was made at a cost of a little less than 3½ cents a pound.

In both cases the results indicate that stockmen are justified in making use of cactus as an efficient and cheap source of nutriment for cattle.

Further studies of cacti, including a large number of chemical analyses from a feed-stuff standpoint, are nearly completed, and the results will be ready for publication in the near future.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Dr. A. D. Melvin,
Chief, Bureau of Animal Industry.

PREFACE.

The evident value of prickly pear as a forage, judging by the experience of many who have fed this material, the urgent demand for information concerning it, and the lack of experimental data from which a reasonable estimate of the food value can be made, rendered experimental feeding highly desirable. The difficulties in conducting such an experiment were manifold. In the first place, it was highly desirable that the cattle used should be accustomed to the feed. The only section of the country in which such cattle could be found was far removed from any experiment station. Trained experimenters who were familiar with cactus feeding were wholly wanting. Fortunately, however, a number of persons who had fed cactus for many years in southwest Texas appreciated fully the value of the information sought and were willing not only to furnish the cattle and provide the feed, but to attend to the details of the feeding and weighing.

In the experiment with dairy cows conditions were such that it was impossible to feed more than two cows experimentally. Yet a careful inspection of the results show that the care with which Mr. Sinclair carried out the details of the work renders the results of great value as an indication of the possible value of prickly pear as a feed for dairy cows. It is shown that a ration producing between 1¼ and 1½ pounds of butter a day cost in the neighborhood of 13 cents when pear, rice bran, and cotton-seed meal was fed.

Although prickly pear is low in nutritive value from the chemical standpoint, the steer-feeding experiment also shows that there is abundant justification of the practices in vogue of preparing cattle for market upon prickly pear and cotton-seed meal. A gain of 1¾ pounds a day at an expense of 3½ cents a pound compares very favorably with feeding results obtained with standard feeds.

W. J. Spillman,
Agriculturist in Charge of Farm Management Investigations.

CONTENTS.

Page.

Introduction 9

The pear fed 9

Prickly pear in the ration of dairy cows 11

  Conditions of the experiment 11

  Method of feeding 12

  Feeding periods 12

    Daily record for Period I 14

    Daily record for Period IV 14

  Comparison of different periods 15

  Cost of feed 16

  Peculiarities noted in feeding 17

  Condition of the animals 17

  Influence of pear on quality of milk 18

Prickly pear in ration of beef cattle 18

  Conditions of the experiment 18

  Method of feeding 19

  Weighing 19

  Shipment and sale of steers 20

  The feeds used 20

  General observations 20

The nature of chopped pear 22

ILLUSTRATIONS.

PLATES.

Page.
1. Cows used in the milking test 12
2. Fig. 1.—Some of the beef cattle used in the feeding experiments. Fig. 2.—Field of prickly pear on the Sinclair ranch 16
3. Fig. 1.—Machinery ready to chop pear. Fig. 2.—Chopped pear ready for feeding. 20

TEXT FIGURE.

1. Diagram showing average yield of milk of cows Nos. 12 and 13 during periods I, II, III, and IV 15

FEEDING PRICKLY PEAR TO STOCK IN TEXAS.

INTRODUCTION.

Bulletin No. 74 of the Bureau of Plant Industry suggested in a brief, popular way some of the more important features of the problem of utilizing cacti as feed for live stock. The present paper is a continuation of that publication, designed to furnish information upon one feature of the problem treated but slightly in the bulletin mentioned.

In the data here presented the aim has been to secure a record of the value of pear as commonly fed. It has not been the main purpose to determine the best methods of feeding this forage plant. In the experiments outlined the endeavor was made to change current practice no more than was necessary to secure the essential data. To determine accurately the value of prickly pear as a dairy or fattening ration would require more elaborate experiments. It has been the aim to give here simply a record of what the rancher realizes from his pear by the ordinary methods of feeding, though such other data as the records have revealed have been noted.

Two experiments are outlined, both conducted under the immediate supervision of ranchers in southern Texas in cooperation with the Bureau of Plant Industry. The first test was undertaken by Mr. Alexander Sinclair, of San Antonio, to whom the greatest credit is due, not only for the conduct of the work but also for assistance in planning the experiments and for suggestions in connection with the interpretation of results. The actual work was performed under his immediate direction by his son, Mr. William Sinclair. The second test was conducted by Mr. T. A. Coleman upon his ranch at Encinal. The feeding was done under Mr. Coleman's immediate supervision, and to his interest and varied experience is due whatever success has been attained.

THE PEAR FED.

There is such confusion in the scientific disposition of the prickly pears that it seems almost hazardous to venture an opinion regarding the proper names of even such common and conspicuous species as those of southern Texas. After studying the forms for two years, however, the writer believes that he can readily determine all the species growing in the region and fed in these experiments. There is, however, such variation in the limitation of the species considered in these pages that it may be advisable to note more than one species in what is here called *Opuntia lindheimeri* Engelm.

There are usually recognized in this region two species of prickly pear, known, respectively, as *Opuntia macrorhiza* Engelm.[a] and *Opuntia lindheimeri* Engelm. The former is a small, prostrate, usually tuberous-rooted species, of no special economic importance. The latter has at least two forms more or less distinct, one with yellow spines and the other with spines red or brown at the base. The yellow-spined form is the typical *Opuntia lindheimeri,* as originally described by Doctor Englemann, and the latter corresponds more closely with what was originally named *Opuntia engelmannii* Salm, although it differs considerably from the typical form[b] of that species as it occurs in the type locality in northern Chihuahua. Both of these forms are at present considered by a majority of botanists to be the same species.

Besides these, there is a form which has also been included under *Opuntia lindheimeri* having spines reddish-brown throughout, with joints somewhat smaller and less prolific at least in a state of nature. This form is also less thorny on the average than the larger-jointed yellow-spined variety, and forms a large part of what is popularly called "blue pear" in southern Texas. However, all blue pear does not have brown spines, for the smoother and more glaucous forms of the yellow-spined variety are also included under this name. All of these forms—those with yellow spines, with brown spines, and with yellow spines brown at the base—have fruits which are normally reddish-purple throughout; but there is a yellow-spined form having green fruits, tinged with purple outside and greenish-yellow within; its seeds also differ very radically from what we consider typical for the species, being about twice as large, the difference in size being made up very largely in the margin. This form is not to be distinguished in any way from the typical yellow-spined form by any habit, spine, or spicule character. Notwithstanding the fact that reproductive characters are supposed to be reasonably constant, the inclination is to consider this also a variety of *Opuntia lindheimeri.*

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[a] It seems better to retain this name until such time as the synonymy of the group can be satisfactorily determined. There is no doubt that the plant in question is the one to which the name was originally applied.

[b] Type specimens when they become old yield but little information regarding the color of the spines, for after being preserved for some time all the spines turn black. This is true of the types of *Opuntia lindheimeri* which have been examined in the herbarium of the Missouri Botanical Garden. Accurate conceptions of these features must therefore be secured by a study of living plants in the type localities.

In addition to those mentioned, there is a distinct species which it is believed has not heretofore been recognized by botanists. This is common south and east of Cotulla, Tex., and consequently is found growing in the Encinal region, where one of the experiments was conducted. It is very distinct from the species previously mentioned, with which it is always associated. It is different in general appearance, as well as in its more strictly botanical characters, being the tallest, most woody, and most loosely branched of the prickly pears of southern Texas. It is characterized by circular joints and by single, erect, long, straw-colored, translucent, bonelike spines, which occasionally have a tinge of red at the base. It blooms and matures its fruit four to six weeks later than the forms of *Opuntia lindheimeri*, the most common of the Texas pears, and its fruit is smaller and more nearly globular. This plant is almost universally known among the Mexican population of this section as "cacanapa." It will doubtless be described as a new species, in which case it would be advisable to use "cacanapa" as the specific name.

All of these forms included under *Opuntia lindheimeri* were used in these experiments, the yellow-spined or typical one predominating in the rations. More of the brown-spined form was fed at Encinal than at San Antonio, although considerable of it was fed at the latter place. At Encinal some cacanapa was fed, but probably not more than 1 or 2 per cent of the ration.

PRICKLY PEAR IN RATION OF DAIRY COWS.

CONDITIONS OF THE EXPERIMENT.

The two cows selected for the experiment were secured from Mr. Sinclair's herd of about 100 head. As it was desirable to have gentle cattle, the selection was made especially with this point in view. They were, however, typical specimens of the herd in other respects and were known upon the ranch as Nos. 12 and 13. They are both Holstein-Jersey stock. In No. 12 Holstein characters predominated decidedly, while in No. 13 Jersey characteristics were more prominent. No. 13 was 6 years old and dropped calf November 27; No. 12 was 7 years old and dropped calf December 6. They were thoroughly accustomed to pear pastures and had been fed singed pear for two to four months each winter. (See pl. 1.)

During the feeding period the cows were kept in separate sheds, opening to the east into small pens about 10 yards square. There were feeding troughs in the sheds and a constant supply of water was kept in the pens. On the whole the sheds were a little more exposed than the barn where the general herd was kept, but the herd was turned out every night except during the coldest weather, while the test cows had their choice of shed or pen.

To accustom the animals to their new quarters they were removed from the herd and put in these pens one week before record keeping was begun. They were perfectly contented from the start. In order to secure uniformity the same person did the milking during the entire experiment.

METHOD OF FEEDING.

The pear was singed in the field with a gasoline torch (see pl. 2, fig. 2), cut and hauled to the barnyard, and unloaded in a pile on the barn floor, from which it was fed as desired. A load was sufficient for a week or ten days. This method kept the pear at a lower, more uniform temperature, no doubt, than that which was fed to the remainder of the herd. The pear in the building during the prolonged cold weather in January did not heat up during the day as much as that standing in the field. The difference, however, was very slight, except during the coldest weather indicated in the tables, when the pear kept indoors was frozen from twenty-four to forty-eight hours longer than that in the field. Each feed was weighed separately at the time of feeding.

At feeding time the material was placed in a box and chopped with a spade into pieces of a convenient size for the animals to eat, usually the equivalent to 2 or 3 inches square. The grain was invariably fed at milking time, and a ration of roughage consisting of pear or sorghum hay, or both, was fed three times each day. Pear was always fed after milking morning and evening, and about midday. It was the purpose during the entire period to feed all the pear the cows would eat, with a definite ration of grain and hay, or of grain alone. There was consequently some pear left in the boxes each morning. This was always cleaned out and deducted from the previous day's ration. It is usual when feeding for beef to sprinkle the meal over the chopped pear, but this could not be done here, for it was the purpose to get as much information as possible regarding the quantity of pear which the animals would consume with a definite grain and hay ration,. or without the latter. The meal could not, therefore, be fed with the pear on account of the waste which would occur and the indefinite character of the results so far as the quantity of grain fed was concerned.

FEEDING PERIODS.

The first period covered twenty days, beginning January 25 and ending February 13. During this period the cows were fed, as they had been during the forty-seven days immediately preceding the experiment, rations consisting of rice bran, cotton-seed meal, a small feed of sorghum hay, and all the prickly pear they would eat. During the next four days the sorghum hay was gradually reduced so that by

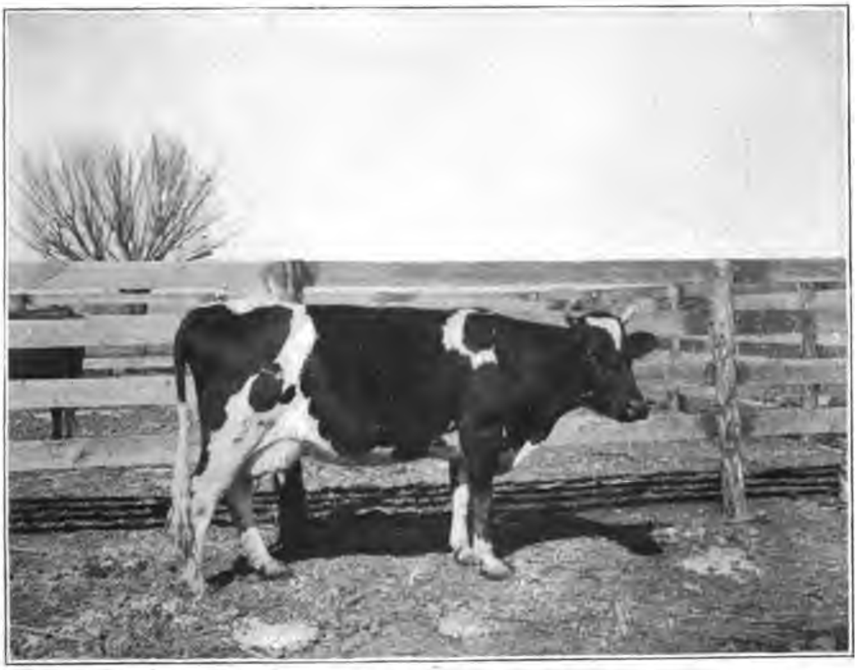

FIG. 1.—COW NO. 12.

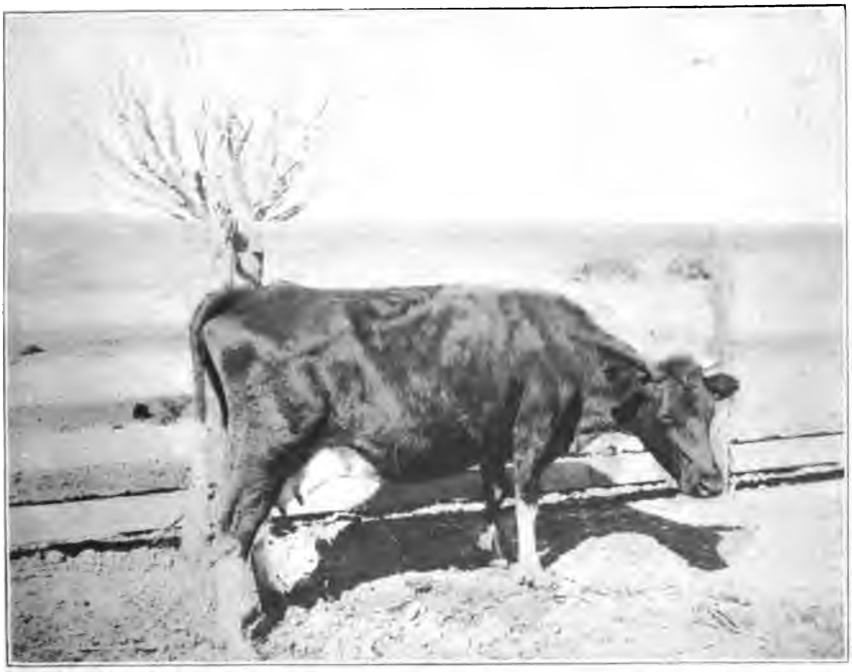

FIG. 2.—COW NO. 13.

COWS USED IN THE MILKING TEST.
Photographed February 22, 1905.

February 18, when the second period began, cactus formed the only roughage fed. Period II extended over eighteen days. During the twelve days immediately following Period II the roughage fed cow No. 12 was gradually changed from cactus to sorghum hay, so that during the third period of the experiment, which lasted fourteen days, the roughage fed cow No. 12 consisted entirely of sorghum hay, while that fed cow No. 13 consisted entirely of cactus. During the seven days between Periods III and IV the roughage of each cow was completely changed, in the case of one from sorghum hay to cactus, and in the other from cactus to sorghum hay. Period IV lasted fifteen days.

It will be noted that these cows at the close of the first period of this experiment had been fed without change of ration for sixty-seven days. During the experiment the roughage fed each cow was changed first to cactus alone, and then to sorghum hay alone. In the case of cow No. 12 the roughage was changed back to cactus alone during the last period. It is notable that the normal milk flow was hardly interrupted during the whole experiment and that the yield of milk was satisfactory throughout, except for a slight decrease just at the close of Period I, evidently due to unusually cold weather.

In the following tabular statements it has been thought wise, since the data are available, to include the daily record for Periods I and IV, inasmuch as this is, we believe, the first published account of pear-feeding data. Ordinarily one period would be sufficient for this, but two are included on account of the excessively low temperatures of late January and early February, introducing variations which would not ordinarily occur. Since the excessively low temperatures influenced results so materially, the United States Weather Bureau observations at San Antonio are incorporated up to February 13, 1905, for convenient reference in interpreting the decrease in milk flow during the first two or three weeks of the experiment.

DAILY RECORD FOR PERIOD I.

During this period the cows were upon the same feed they had been accustomed to at this time of the year. They had been fed this ration since December 9. Besides the feed tabulated below, cow No. 12 got 12 pounds of rice bran and 3 pounds of cotton-seed meal, with the exception of the last two days of the period, when she would eat only 11 pounds of rice bran. Cow No. 13 was started in at 12 pounds of rice bran and 3 pounds of cotton-seed meal, but the rice bran was decreased to 10 pounds on January 28 and to 8 pounds on February 6, because 8 pounds of rice bran was all that she would clean up. It was the purpose to feed all the sorghum hay and pear that the cows would eat during this period.

Date.	Yield of milk.		Butter fat in milk.				Amount of roughage fed.				Atmospheric temperature.	
			Per cent.		Amount.		Prickly pear.		Sorghum.			
	Cow No. 12.	Cow No. 13.	Cow No. 12.	Cow No. 13.	Cow No. 12.	Cow No. 13.	Cow No. 12.	Cow No. 13.	Cow No. 12.	Cow No. 13.	Maximum.	Minimum.
1905.	*Lbs.*	*Lbs.*			*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	°*F.*	°*F.*
January 25	38.0	33.0	3.8	4.0	4.28	3.78	60	62	12	15	44	31
January 26	37.0	30.0					86	90	10	10	41	25
January 27	37.5	31.5					101	101	10	10	45	38
January 28	40.0	32.5	3.6	4.0	....	3.88	116	116	8	8	68	43
January 29	43.0	32.5					125	125	8	8	60	44
January 30	40.6	33.0					125	125	8	5	52	36
January 31	39.9	31.8	3.6	4.0	8.75	3.76	135	130	8	5	45	49
February 1	41.2	33.3					138	137	8	4	58	34
February 2	38.5	28.9					142	139	8	5	34	29
February 3	38.6	29.1	3.8	4.2	5.53	4.52	139	149	8	5	33	27
February 4	36.4	27.3					81	88	8	5	27	21
February 5	34.6	25.9					103	105	8	5	41	25
February 6	35.9	25.3					117	138	8	5	32	23
February 7	36.5	26.8	3.9	4.2	5.83	4.88	113	139	8	5	34	29
February 8	38.5	29.5					118	133	8	2	60	32
February 9	38.8	29.8					98	124	8	3	61	37
February 10	35.7	30.3					89	109	8	3	51	37
February 11	37.0	31.0	4.2	4.3	4.3	3.54	100	114	8	4	56	44
February 12	33.8	27.5					89	96	8	5	53	17
February 13	[a] 31.9	[a] 24.0					90	92	[b] 12	[b] 12	35	13

[a] The rapid decrease in milk flow toward the close of this period is doubtless due to the unusually low temperatures.

[b] Extra sorghum hay fed on account of frozen pear.

DAILY RECORD FOR PERIOD IV.

Period IV covered 15 days, with cow No. 12 receiving all the pear and cow No. 13 all the sorghum hay they would eat. Cow No. 12 received 12 pounds rice bran and 3 pounds cotton-seed meal and cow No. 13, 8 pounds rice bran and 3 pounds cotton-seed meal.

Date.	Yield of milk.		Butter fat in milk.				Amount of roughage fed.			
			Per cent.		Amount.		Prickly pear.		Sorghum.	
	Cow No. 12.	Cow No. 13.	Cow No. 12.	Cow No. 13.	Cow No. 12.	Cow No. 13.	Cow No. 12	Cow No. 13.	Cow No. 12.	Cow No. 13.
1905.	*Lbs.*	*Lbs.*			*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
April 10	33.6	27.7	3.6	3.8	2.49	1.98	141	....	....	19
April 11	35.7	24.4					149	....	....	26
April 12	36.8	28.6	3.8	3.9	1.39	1.11	150	....	....	23
April 13	36.2	28.4	3.4	3.2	1.23	.90	160	....	....	22
April 14	36.0	27.8	3.2	3.6	1.15	1.00	165	....	....	22
April 15	32.6	27.5	3.6	3.8	1.17	1.04	119	....	....	21
April 16	32.4	28.4	3.6	4.2	1.16	3.47	[a] 90	....	....	23
April 17	30.3	27.0			1.09		120	....	....	25
April 18	31.1	27.4			1.11		116	....	....	23
April 19	31.6	27.5	4.0	3.8	1.26	3.19	143	....	....	25
April 20	33.9	29.4			1.35		158	....	....	23
April 21	32.2	27.3	3.6	3.8	1.15		154	....	....	22
April 22	34.4	28.4	3.4	4.0	1.16	1.13	160	....	....	24
April 23	33 1	26.6	3.8	3.7	1.25	2.03	174	....	....	23
April 24	33.5	28.3			1.27		172	....	....	25
Average	....	....	....	....	....	....	149	....	....	23

[a] Young joints not eaten not counted in average.

COMPARISON OF DIFFERENT PERIODS.

The following table shows the milk and butter yield of the different periods:

Period.	Cow No. 12.			Cow No. 13.		
	Feed.	Average daily yield of milk.	Average daily yield of butter.[a]	Feed.	Average daily yield of milk.	Average daily yield of butter.[a]
		Pounds.	*Pounds.*		*Pounds.*	*Pounds.*
I	Pear and sorghum	37.6	1.68	Pear and sorghum	29.6	1.43
II	Pear	35.7	1.46	Pear	29.1	1.36
III	Sorghum	33.7	1.46	Pear	29.4	1.32
IV	Pear	33.5	1.43	Sorghum	27.6	1.24

[a] In computing butter yield, 0.85 pound of butter fat is considered equal to 1 pound of butter.

If we compare an average of Periods II and IV with period III for cow No. 12 we have an average daily yield of 34.6 pounds of milk and 1.445 pounds of butter on pear, and an average of 33.7 pounds of milk and 1.46 pounds of butter on sorghum. In the case of cow No. 13 the periods can not be so satisfactorily grouped to eliminate the effect of advancing lactation. The best comparison that can be made is between an average of Periods I and IV and II and III, when the record shows the following:

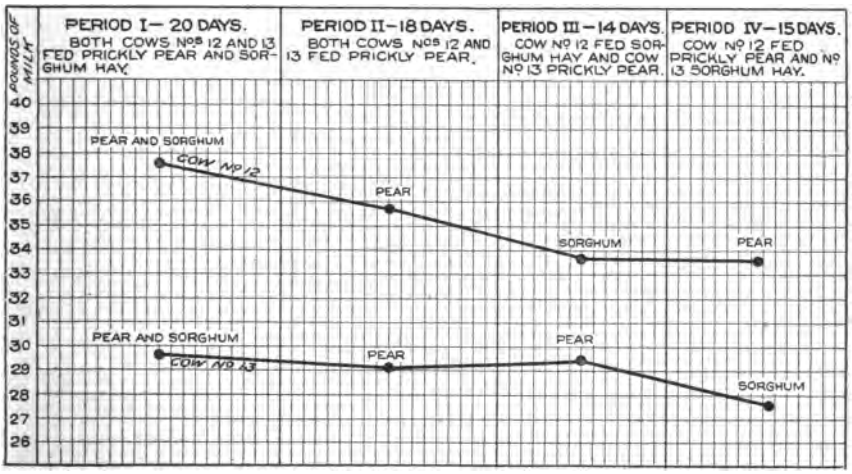

FIG. 1.—Diagram showing average yield of milk of Cows Nos. 12 and 13 during Periods I, II, III, and IV. The character of the roughage is indicated for each animal in each period. The scale showing the yield of milk in pounds per day is placed at the left. The small circles indicate the average yields for the periods. It will be noticed that the decline in yield, which is to be expected as lactation advances, is not quite so rapid on pear as it is on sorghum hay.

Average for Periods I and IV (sorghum mostly), 28.6 pounds of milk and 1.335 pounds of butter.

Average for Periods II and III (pear), 29.25 pounds of milk and 1.340 pounds of butter.

The relative milk flow can be appreciated more readily in the accompanying diagram (fig. 1).

The tables and the diagram show:

(1) Cow No. 12 shows a gradual decrease from a pear and sorghum ration in Period I to a pear ration in Period II, but not quite so rapid a decline as took place during Period III, when sorghum was fed. In Period IV, while a decrease is shown, it is less marked than in the preceding sorghum period.

(2) Cow No. 13 shows a slight decline in Period II and an almost complete recovery in Period III, but a sharp decline when sorghum is fed in Period IV.

(3) A full roughage ration of pear with a constant grain ration appears to yield fully as good results as a full roughage ration of sorghum hay. The records are really a little more favorable to the pear ration.

COST OF FEED.

It is impossible with our present imperfect knowledge regarding the rate of growth and habits of prickly pear under cropping conditions to make an estimate which is at all reliable regarding the cost of this item of the ration. In the computations, therefore, it is deemed best to omit the item of cost of producing the crop of pear. The estimates do not, therefore, contain any account of the use of the land upon which the pear is grown. At all events, this would be in accord with the general sentiment that pear costs nothing. This, of course, is not strictly true, although the rancher has as yet paid but little attention to prickly pear culture. He gathers it from his native pastures as he does his firewood. Upon this farm, however, a considerable effort has been made to propagate the plant, though the cost of the effort could not be estimated. The cost of the other items of the ration was as follows, the prices quoted being those actually paid upon the ranch during the time the feeding was in progress:

	Per ton.
Cotton-seed meal	$22
Rice bran	13
Sorghum hay	7

One man can easily burn pear for 100 cows, and in addition he can assist in milking. He will use about 10 gallons of gasoline each day. During the past winter this cost 12 cents a gallon. The cost of a day's rations for each cow while pear without hay was being fed was as follows:

	Cents.
12 pounds of rice bran	7.8
3 pounds of cotton-seed meal	3.3
Labor	.75
Gasoline	1.2
Total	13.05

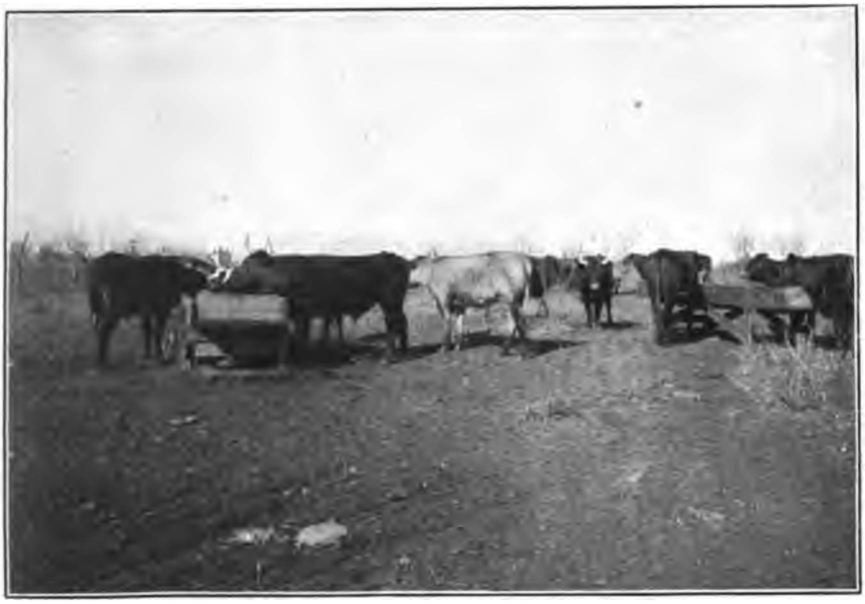

FIG. 1.—SOME OF THE BEEF CATTLE USED IN THE FEEDING EXPERIMENTS.

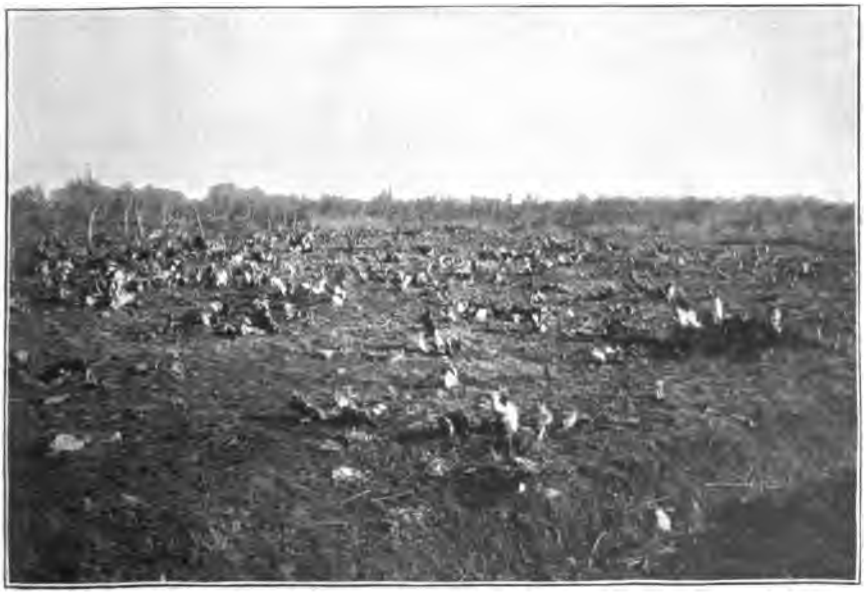

FIG. 2.—FIELD OF PRICKLY PEAR ON THE SINCLAIR RANCH.

This estimate is a trifle high, as it includes the cost of labor in excess of the time actually occupied in feeding. Thirteen cents a day will, therefore, represent very closely the entire cost of a ration as outlined above. When sorghum hay was fed in addition to the pear the cost of the feed was a little higher, but as hay was increased the cost of labor and gasoline decreased. It must be remembered, also, that the above estimate of 13 cents represents the maximum cost of the ration of the test cattle, and that the computation of the cost of feeding pear is based upon actual experience on the ranch during the past several years.

PECULIARITIES NOTED IN FEEDING.

On April 15 and 16, when cow No. 12 alone was on a full roughage ration of pear, it was observed that she left more than usual in the trough, although she seemed to relish the feed. This was at the time when young joints were first fed in any quantity, and it was soon discovered that it was pieces of these and not of the older joints that were left. After this the young joints were thrown out and no more of them fed during the remainder of the experiment. In the field cattle eat these young shoots readily in the spring, while they may not molest the older ones, but the reason is probably due to the condition of the spines alone. They would probably eat the older joints even more readily than the younger ones were they not so formidably protected.

The leathery texture of the young joints appears to be responsible for the fact that the cow refused to eat them when more palatable material was fed. All who have worked with prickly pear, especially botanists who have attempted to prepare specimens, have noticed that the young joints are very tough and leathery. Indeed, it is with considerable difficulty that one is able to split a young joint lengthwise with a knife, while the older ones are very easily cut.

It was a constant surprise to observe the fondness of the cattle for the singed pear. During the latter part of the first period the temperature was unusually low for southern Texas. The United States Weather Bureau records show a maximum of only 35° and a minimum of 13° F. on February 13. It will be seen that a little extra sorghum hay was fed on this day. Regardless of the fact that the pear was frozen solid all day the cows ate 90 and 92 pounds, respectively. This was the coldest day of the winter, but not the only day when the cows ate frozen pear with apparent relish.

CONDITION OF THE ANIMALS.

The distance of the ranch from any convenient means of weighing prevented the securing of data on the important point of the weight of the cows, but careful observations were made by several individuals, both those having immediate charge of the animals and those who saw them only occasionally. All agreed that the condition of both cows continued to improve up to the end of the experiment.

Of course, the well-known laxative effect of prickly pear was evident during the entire time that it was fed, being less noticeable while sorghum was a part of the ration; but at no time was it thought that the cows scoured to any injurious extent, even during the period when pear was the only roughage fed them. The fact that they apparently gained in flesh, milked well, and began shedding earlier than the general herd appears to be sufficient proof that they were in good physical condition during the entire period.

INFLUENCE OF PEAR ON QUALITY OF MILK.

The statement has frequently been made that the quality of milk is injuriously affected when pear is fed to dairy cows, and it seemed important to secure data on this point in connection with this experiment. Mr. Sinclair has fed pear to his herd for two to four months each year for six or eight years, and no complaint has ever been received from customers which could in any way be attributed to pear feeding.

During the time when one of the cows was on a full roughage ration of pear—that is, on rice bran, cotton-seed meal, and pear with no sorghum hay—five persons tested the milk to determine whether any odor or flavor was imparted by such a ration. Morning's milk was examined in the evening with the result that four persons could not detect any change, deleterious or otherwise, while one was in doubt.

PRICKLY PEAR IN RATION OF BEEF CATTLE.

CONDITIONS OF THE EXPERIMENT.

As stated previously, an effort was made to keep the steers fed in the beef experiment under conditions as nearly similar to those prevailing in the general pear-feeding region as possible. The steers selected were from the general Coleman herd, a miscellaneous lot, a majority of which were bred near Cactus, Tex. They were considerably above the average of the cattle in the neighborhood, or even on Mr. Coleman's ranch. (See pl. 2, fig. 1.)

The intention was to feed one carload (20 head) of steers, but when the animals were gathered 27 head were weighed and put in the pen. The additional 7 head were not removed until the close of the experiment, but only the original carload of 20 head was shipped at the close of the feeding, the others being shipped with a miscellaneous lot of cattle to another market.

The feeding lot was an ordinary open mesquite "trap," containing approximately 4 acres of ground, and inclosed by a wire fence. No shelter of any kind was furnished the cattle. The scrub mesquite brush in and surrounding the feed lot offered very little protection. This might not be a serious consideration in an average southern Texas winter, but during the past winter protection would, have enhanced very considerably the gains made.

METHOD OF FEEDING.

The method of feeding in this case was exactly that employed throughout the pear region of Texas wherever the pear chopper is used. The largest and most woody plants available were used, from localities where the growth was most vigorous and healthy. They were chopped with one of the common pear choppers, but without singeing.

In this experiment the feed was gathered from the field twice each day—at about 7 o'clock in the morning and 3 o'clock in the afternoon—cut, and fed immediately. The chop was shoveled into the ordinary feeding troughs, and the cotton-seed meal was sprinkled upon it in such quantity as would give the desired number of pounds for each animal.

With this method of feeding it was not feasible to furnish more pear than the animals would eat, because of the necessary waste of meal, but a constant effort was made to give them all they would clean up.

WEIGHING.

Although the steers used were probably more gentle than the average stockers of southern Texas it was found impracticable to secure weekly weighings, as was the intention in the beginning. The two weighings that were made, it is believed, cost the gains of an entire week. All the animals became considerably excited, and once or twice threatened to stampede.

On account of the apparently good gains being made by this lot of steers Mr. Coleman decided to put another herd of 100 head on feed in an adjoining pen. At first these also did very nicely, but they soon became wild, with no apparent cause, and it was decided to turn them out into pasture again.

The experimental lot did not get nearly as wild as the others, even with the weighing, but there is no doubt that the final gains were very materially reduced by the excitement caused during the weighing. It should be stated that the greatest care was exercised by Mr. Coleman in the handling of these steers during the entire period. Aside from the necessary handling and weighing they were subjected to no circumstances to excite them.

SHIPMENT AND SALE OF STEERS.

On the evening of April 28 the final weighing was made and the steers, after receiving about a one-half ration on the morning of April 29, were driven into the stock pens at Encinal and loaded into a car. They did not leave the yard, however, until the following morning. They were consigned to Fort Worth, where they were sold at $4.25 per hundredweight on a break in the market on May 2. The account sales showed 21,560 pounds to be the weight of the 20 steers, giving a loss in transit of 1,770 pounds, or 88½ pounds for each animal. This shrinkage is not considered excessive by Mr. Coleman, whose records of shipments from the ranch during past years are probably not excelled for accuracy by those of any ranch in Texas. In some shipments the shrinkage has been greater and in others less than in this instance in the case of steers shipped off the range.

THE FEEDS USED.

The following table shows the kinds and quantities of the feeds used by periods:

Date.	Days in each period.	Total pounds of pear.	Average pounds of pear per head per day.	Total pounds of cotton-seed meal.	Average pounds of cotton-seed meal per head per day.
Jan. 15-Jan. 21	7	16,890	89.36	220	1.16
Jan. 22-Jan. 28	7	17,567	92.94	300	1.59
Jan. 29-Feb. 3	6	15,195	93.79	350	2.16
Feb. 4-Feb. 10	7	18,345	97.06	700	3.70
Feb. 11-Feb. 17	7	15,625	82.67	700	3.70
Feb. 18-Feb. 25	8	20,395	94.42	800	3.70
Feb. 26-Mar. 4	7	19,040	100.74	1,050	5.55
Mar. 5-Mar. 11	7	19,655	103.99	1,050	5.55
Mar. 12-Mar. 18	7	19,975	105.69	1,050	5.55
Mar. 19-Mar. 25	7	19,315	102.19	1,050	5.55
Mar. 26-Apr. 1	7	18,840	99.68	1,050	5.55
Apr. 2-Apr. 8	7	18,715	99.02	1,050	5.55
Apr. 9-Apr. 15	7	18,850	99.74	1,050	5.55
Apr. 16-Apr. 22	7	19,175	101.45	1,050	5.55
Apr. 23-Apr. 28	6	16,275	100.46	900	5.55
Apr. 29	1	2,100	77.77	80	2.96
Average			96.31		4.31

GENERAL OBSERVATIONS.

In spite of the very unfavorable weather, and adverse conditions generally, the gains made were comparatively satisfactory. Of course the gains were not as great as those of stall-fed cattle, nor even those obtained in the older and better established feeding sections. However, the cost of a pound of grain, computed from the above tables and data, is more favorable to the combined cotton-seed meal and prickly-pear ration than one unfamiliar with prickly pear as a roughage would suppose. On an average 77.6 pounds of prickly pear and 2½ pounds of cotton-seed meal produced 1 pound of gain;

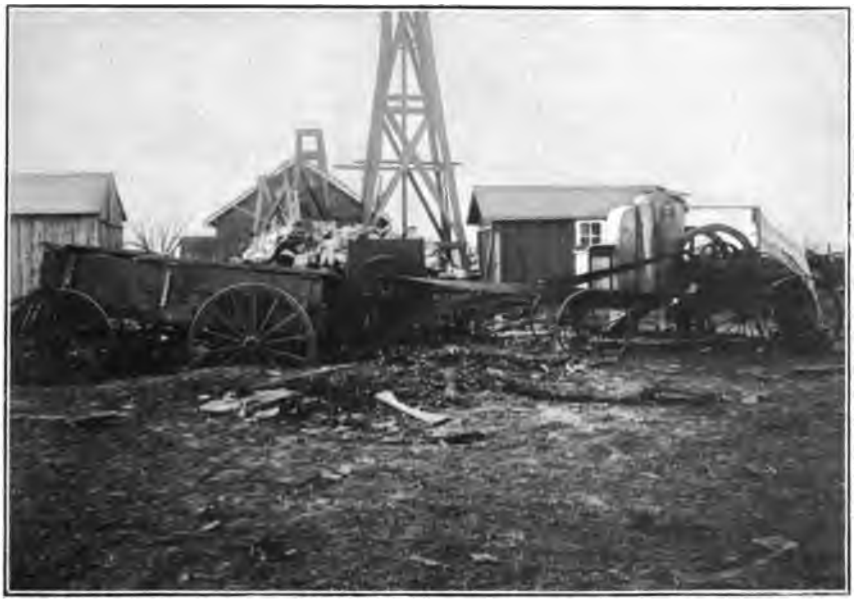

FIG. 1.—MACHINERY READY TO CHOP PEAR.

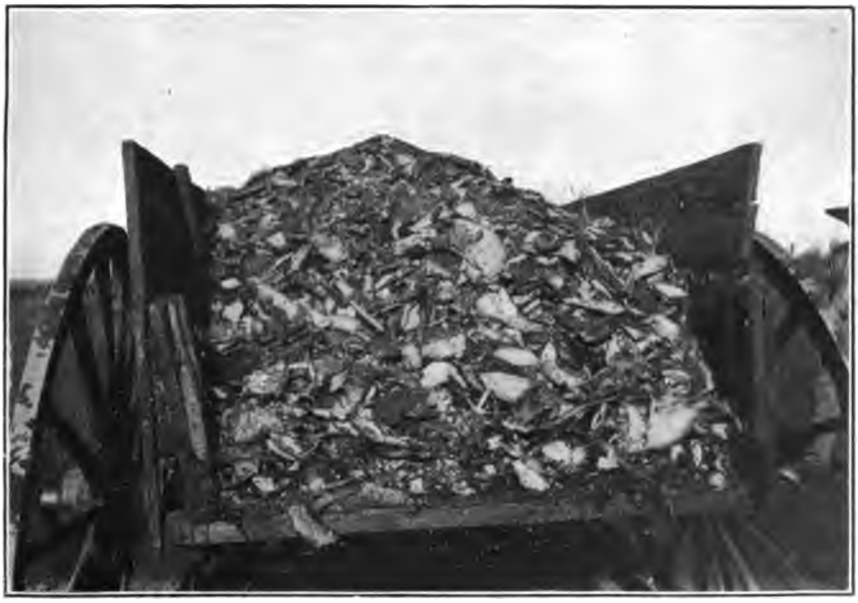

FIG. 2.—CHOPPED PEAR READY FOR FEEDING, ON THE COLEMAN RANCH.

or, in actual outlay of cash for feed at the prevailing price for meal of $23.75 per ton, 1 pound of gain cost 2.97 cents' worth of cottonseed meal, which is not at all excessive for the cost of grain to feed with pear.

The cost of labor can not be accurately determined for this experiment because of the small number of animals which were fed, but the data furnished here, together with the experience of Mr. Coleman and others in feeding pear during the past ten years, enables one to make a very close estimate of the necessary expenses. The actual conditions were that one man did all of the feeding during the entire period, and was assisted in the chopping by three other men—an engineer and two laborers. He in return assisted them in chopping two loads for each one that he used, and their loads represented about 50 per cent more pear than his. All pear was hauled an average distance of 1 mile, and each load was weighed on the way from the field to the chopper, necessitating a little extra travel. While all that was required of the man in charge of the feeding was the care of these animals, his time was not entirely occupied. Indeed, it is believed he would have had little difficulty in feeding 100 head under these conditions. In actual practice much less labor would be required, both on account of greater convenience in feeding and greater economy of time.

In Bulletin No. 74 of the Bureau of Plant Industry estimates are made which indicate that eight men can feed a maintenance ration to 1,200 head of cattle. Reducing this number to the extent necessary to compensate for the additional care required in the feeding of a fattening ration, it is estimated that eight men could without doubt feed 1,000 head of cattle. Assuming the figures of cost in the publication mentioned to be correct, the total expense of labor, gasoline, and interest on machinery would be in the neighborhood of 90 cents for each animal for a period of one hundred days.

The value of the pear is not included in this estimate, and, as in the previous experiment, it was not possible to determine its cost. Should one ask a rancher in southern Texas to estimate upon this point, his answer would invariably be, "Nothing." In fact, it is questionable whether the pastures are not actually improved by cutting off the older, larger plants. As in all fattening experiments, the increase in weight alone does not represent the entire gain; the improvement and enhanced valuation of the whole carcass must be taken into consideration, but all of the estimates are based upon value of the increased weight alone.

The relation of gain to feed consumed may be summed up as follows:

1. Average daily ration of pear for each head of stock, 96.31 pounds.

2. Average daily gain for each head, 1.75 pounds.
3. Amount of pear fed for 1 pound of gain, 55.03 pounds.
4. Amount of cotton-seed meal required for 1 pound of gain, 2½ pounds.
5. Cost of cotton-seed meal for 1 pound of gain, 2.97 cents.
6. Cost of pear per 1 pound of gain 0.514 cent.
7. Cost of feed per 1 pound of gain, 3.48 cents.

THE NATURE OF CHOPPED PEAR.

Since many erroneous statements have appeared regarding the nature of pear chop, and since the publications of the Department on the subject have been misinterpreted, this seems to be a fitting place to put in a few words of explanation regarding the work of the pear chopper and the character of the feed produced.

A description of the pear choppers is given in a previous bulletin[a] and need not be repeated here. (See also pl. 3, fig. 1.) The construction of the machine indicates that the pear may be reduced to very fine consistency. But pieces 6 inches square may be found in the chop when ready to feed. Plate 3, figure 2, shows this condition fairly well. The material is there represented in the rear end of a wagon as it was thrown out of the machine by the centrifugal force of the revolving wheel (pl. 3, fig. 1). Large pieces are shown; but no special injury to the cattle was observed from feeding them. It is evident that all pear joints fed to the machine at right angles to the knives, as described in the publication referred to above, will be cut into pieces ½ to 1½ inches in length, depending upon the setting of the shear plate; but whatever material happens to be fed in such a way as to reach the machine in the plane of the knives will pass through in large flat pieces. Often a piece of joint 4 to 6 inches square, or even a whole joint, will pass through the machine with practically an uninjured epidermis. The material never is macerated or reduced to a pulp. In spite of this, however, little or no evil effect results from the spines, even in the case of joints which pass through the machine uncut. The dead and exceedingly brittle spines have invariably received enough rough treatment in passing through the machine to reduce very perceptibly the injury which they can do. There is no denying the fact that stock which are fed pear chopped in this way are somewhat annoyed by the spines. There is always more or less slobbering as the result of the spines sticking into the membranes of the mouth, but the effect does not appear to be a serious one.

It has been frequently stated that the spines are softened by the juices of the plant in the chopped material to such an extent as to render them innocuous—an idea which is entirely erroneous. It is always the practice to feed immediately after chopping. Indeed, it is doubtful whether the spines would become very materially softened before the chop would ferment to such an extent as to render it unfit to feed. The effect upon the spines is entirely one of abrasion; they are broken to such an extent that the injury they cause is very much reduced. Cattle can handle pretty rough feed, and they eat much of the Texas pear as it stands in the pastures. It is believed that if the spines lay tightly against the surface of the joint, instead of approximately at right angles to it, the cattle could graze the pear with but little difficulty. It should be emphasized that in pear chopping the spines are not softened by the juices and the material is not macerated, but that the chances of the spines doing injury are reduced to a minimum by the rough treatment which they receive from the machine. It is also evident to anyone watching the operation of a pear machine that many of the spines are winnowed out and removed from the product during the process of chopping.

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[a] Bulletin No. 74, Bureau of Plant Industry, p. 17, Pls. II and III.

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Issued January 29, 1907.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN NO. 92.

A. D. MELVIN, CHIEF OF BUREAU.

GENERAL LI
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MAR 1 1907

THE MILKING MACHINE AS A FACTOR IN DAIRYING.

(A PRELIMINARY REPORT.)

I.—PRACTICAL STUDIES OF A MILKING MACHINE.

BY

C. B. LANE, B. S.,

Assistant Chief, Dairy Division, Bureau of Animal Industry.

II.—BACTERIOLOGICAL STUDIES OF A MILKING MACHINE.

BY

W. A. STOCKING, JR., M. S. A.,

Bacteriologist, Storrs Agricultural Experiment Station,
Professor of Dairy Bacteriology, Connecticut
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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., October 13, 1906.

Sir: I have the honor to transmit herewith a preliminary report on "The Milking Machine as a Factor in Dairying," an investigation of this subject by the Dairy Division having been authorized by you. The first part deals with the practical side and the second part with the bacteriological phase of the subject.

Recent improvements in milking machines have led to their introduction and practical use in some of the larger dairies of the country, and there are indications that these machines may come into general use in the near future and become an important economic factor in the dairy industry. While the investigations so far made have been too limited to justify any sweeping deductions or positive conclusions, it is believed, in view of the importance and present interest of the subject to dairymen, that the results here presented are of sufficient value to warrant their publication, and I therefore recommend the publication of this report as Bulletin No. 92 in the series of this Bureau. The investigations will be continued and further results may be reported later.

The experimental work which is the basis of this bulletin was conducted in August, 1905, but owing to unavoidable delays the manuscript has not been ready for publication until now.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

I.—PRACTICAL STUDIES OF A MILKING MACHINE.

Page
Introduction 9
Some milking machines in use 10
  A foot-power milker 11
  A power milker 11
  A milker designed for either hand or power 12
  The machine used in the investigations 12
Cost of equipment for machine milking 13
Different kinds of power which may be utilized 13
Experiments in which hand and machine milking were compared 14
  Experiment No. 1 14
    Time required for hand and machine milking compared 17
    Yield of milk from hand and machine milking compared 18
    Thoroness of hand and machine milking compared 20
  Experiment No. 2 20
    Time required for hand and machine milking compared 24
    Yield of milk from hand and machine milking compared 24
    Thoroness of hand and machine milking compared 25
    Quality of the milk 26
General considerations 27
  Effects of milking machines upon the cows 27
  Adjusting the machines to the cows 28
  Practicability of using machines for large and small herds 29
  How the general introduction of the milking machine would affect the dairy industry 29
  Suggestions for improvements 29
  Objections and difficulties 30
  Need for further investigations 31
Results of experience with milking machines as reported by dairymen 32
Addendum 32

II.—BACTERIOLOGICAL STUDIES OF A MILKING MACHINE.

Production of sanitary milk 33
Experiments designed to test the sanitary character of machine-drawn milk 34
  Experiments at Farm No. 1 34
    Method of experiment 34
    First treatment of the milking machines 36
    Second treatment of the machines 37
    Third treatment of the machines 40
    Fourth treatment of the machines 41
    Fifth treatment of the machines 41
    Sixth treatment of the machines 44
    Keeping qualities of the milk 44
    Summary of experiments at Farm No. 1 45

Experiments to test the sanitary character of machine-drawn milk—Cont'd. Page.

Experiments at Farm No. 2 46
  Method of experiment 46
  First treatment of the machines 47
  Second treatment of the machines 48
  Third treatment of the machines 49
  Fourth treatment of the machines 50
  Fifth treatment of the machines 52
  Sixth treatment of the machines 52
  Keeping qualities of the milk 54
Summary of results of bacteriological investigations 54

ILLUSTRATIONS.

PLATES.

Page.

PLATE 1. Fig. 1. A foot-power milker with attachments. Fig. 2. The same machine in operation........ 12

2. Fig. 1. A power milker with attachments. Fig. 2. The same machine in operation........ 12

3. Fig. 1. Milking machine for use with either hand or power. Fig. 2. Same machine in operation........ 12

4. Fig. 1. Milking machine used in experiments. Fig. 2. The same machine in operation........ 12

TEXT FIGURES.

FIG. 1. Bacteria in atmosphere of barn at farm No. 1........ 35

2. Bacteria in machine-drawn milk in experiment No. 37........ 39

3. Bacteria in machine-drawn milk in experiment No. 101........ 40

4. Bacteria in milk drawn by steam-sterilized machine in experiment No. 101A........ 42

5. Bacteria in machine-drawn milk in experiment No. 103........ 43

6. Bacteria in atmosphere of barn at farm No. 2........ 46

THE MILKING MACHINE AS A FACTOR IN DAIRYING.

I.—PRACTICAL STUDIES OF A MILKING MACHINE.

By C. B. LANE, B. S.
Assistant Chief, Dairy Division, Bureau of Animal Industry.

INTRODUCTION.

For more than a quarter of a century machinery has been successfully used in separating the cream from the milk and churning it into butter, but a machine for milking the cows has been more difficult to supply. It has long been realized that a successful and practical cow milker would mean a profitable revolution of the dairy industry, as it would render the work of milking much easier and reduce the necessity for hired help, thus making the dairyman more independent. Efforts have been made by scores of inventors for more than half a century to construct a machine that would milk cows in a satisfactory manner and without injury. The German writer Martiny states that 29 different milking machines known to him had been patented in different countries or mentioned in the dairy literature between the years 1877 and 1898.

The annual reports of the United States Commissioner of Patents show that during the period of 34 years from 1872 to 1905, inclusive, 127 patents were taken out in this country alone for milking machines or separate parts of them. A number of machines have been successful in extracting the milk from the cow by either pressure or suction, or by the two combined, but have fallen short of being practical in some vital point. Naturally, inventors have attempted to imitate the way in which the calf sucks its dam. The difficulty has been to reproduce the peculiar influence which the sucking calf has upon the cow and to devise a machine which will not irritate the animal and which will do its work without injury. Another difficulty in devising a cow milker has been to construct it so that it could be adjusted to all cows. Individual animals vary greatly in the size and conformation of their udders, and even the same cow varies somewhat in the size and shape of her udder and teats during the different stages of her lactation period. Cows also vary greatly in disposition and temperament. A successful cow milker, therefore, must meet all these different conditions. Since milking must necessarily be an artificial process, it has been thought for some time that animals would become accustomed to being milked by machinery just the same as they become accustomed to hand milking, provided a milking machine could be perfected that would be comfortable to the animal when in operation. A heifer, for example, coming into milk the first time would take as readily to one artificial method as to another, other things being equal.

While no milking machine yet invented has shown its practical value in a way that has led to its general use, recent improvements in machines of this type have resulted in greater simplicity of construction and effectiveness in operation, and consequently they are in practical use in a limited number of the larger dairies. Indeed, the prospect for the general introduction of milking machines appears to be so favorable that it was thought advisable by the Secretary of Agriculture to authorize an investigation of the fundamental problems involved in the use of such machines. These fundamental problems may be stated as follows: (1) The practicability of substituting machine for hand milking in the actual operations of dairying; (2) the effects of machine milking on the quantity and composition of the milk yielded by the cows; (3) the effects of machine milking on the cleanliness, sanitary character, and keeping qualities of the milk.

In making such an investigation it was, of course, necessary to select some machine for use. The machine selected is one which was found to be in actual use in a number of dairies, and one which appeared to offer adequate means for conducting the proposed investigation. Its selection was not intended as in any sense a recommendation of this particular machine to the favor of dairymen. It is believed, however, that, in so far as the investigation has demonstrated the merits of this machine, it will benefit all inventors and manufacturers of successful milking machines.

The authors fully appreciate the fact that these investigations have been too limited to justify any sweeping deductions or positive conclusions. But in view of the probable general introduction of such machinery in the near future, it is believed the results of the investigations are of sufficient value to justify their publication.

SOME MILKING MACHINES IN USE.

As a complete account is hereinafter given of the machine used in the investigation, a brief description will suffice for the other machines presented here, none of which were used in this investigation, and in regard to the efficiency of which no opinion can consequently be exprest.

A FOOT-POWER MILKER.

This machine (pl. 1) is designed for use in small herds and has no stationary fixtures. It consists of a suction pump worked by foot power, 2 pieces of rubber hose, and 8 suction cups to be attached to the teats of the 2 cows, which can be milked at the same time. The milk passes thru the cylinder, and also thru the valve in the pump piston itself. The operator sits between the 2 cows and works the pump with his feet. On opening the spigot the suction rapidly draws the cups over the teats and the milk begins to flow into the milk pail, which is hung on the spout of the pump.

The teat cups are hollow and conical. Nearly an inch from the large end the cup is almost closed by a soft rubber diaphragm; this disk, being elastic, fits air-tight around the different-sized teats. The teats fill the conical cup except at the small end where suction is applied. The cup is made of three pieces of smooth hard rubber. To the end of the cup is attached a piece of glass tubing thru which the milk may be seen, and this is again connected with a small rubber tube. By means of a spigot in the tube the suction may be cut off when the teat is empty. The milk is conveyed from the spigot to the head where the milk from all four teats unites and passes into the large hose which carries it to the pail.

This machine has been in operation since 1892.

A POWER MILKER.

The accompanying illustration (pl. 2) shows a cow milker which has recently been put upon the market. It consists of an ordinary milk pail made of block tin and holding about 15 quarts. On top of this pail is a tight-fitting lid of aluminum. On this lid is mounted a pump or pulsator which works automatically and causes the intermittent action of the machine. Connections are made by means of rubber tubing to the exhaust and air-pressure pipes, which are laid thru the stable with convenient branches between the cows. Two rubber tubes, each about 3 feet long, are also connected with convenient nozzles on the lid, and on the other end of each are 4 cups which fit snugly over the cow's teats, 2 cows being milked into one pail. As the pulsator oscillates (at the rate of about 60 times a minute) the vacuum is alternately turned on and off, the teat cups causing suction and release at each alternate stroke.

The machinery for operating the pulsator consists of an exhaust pump and a compressor; the exhaust produces the suction and operates the pulsator in one direction, while the compressor operates the pulsator in the opposite direction.

A MILKER DESIGNED FOR EITHER HAND OR POWER.

The milking machine shown in Plate 3 consists of a simple air pump, composed of two cylinders, each of which is independent of the other in its action. One cylinder milks one cow, and one the other. The valve chambers, supported at the ends of the rods, are for the purpose of keeping the milk from running back into the pump, and also to give the pump sufficient and continued suction for the space of about ten to fifteen seconds. When the pressure is off of one of these valve chambers the milk flows from it of its own gravity into the pail. Each cow can be milked separately, or both can be milked into one pail, as desired. Cows can be milked into either open or closed pails. The machine is operated by either hand or power, the hand machine being convertible into a power machine by simply bolting an air device to it. In the operation of the power machine it is necessary to pipe the stables, a comprest air tank being required, which must be filled by some power running an air compressor. There are no pulsators or vacuum pumps in the construction of the machine. The teat cups are provided with a rubber sleeve.

THE MACHINE USED IN THE INVESTIGATION.

With this machine the milk is drawn by intermittent suction. The suction may be created by either a vacuum pump or a steam ejector. Connected with the vacuum pump is a vacuum reservoir and a pipe running the whole length of the cow stable, with a connection valve or vacuum cock between each pair of cows. A safety valve is connected to the reservoir to prevent the vacuum from running higher than is desired.

The machine itself (pl. 4, fig. 1) consists of a heavy tin pail, which is cone-shaped and holds about 55 pounds of milk. The cover of this pail is a disk, in which is a vacuum motor which produces the pulsations in drawing the milk from the teats. The cover fits the pail tightly and excludes all air.

To operate the machine it is placed between the pair of cows to be milked. A rubber tube connects the pail top or pulsator with the vacuum cock above the stanchions. On opening the cock the air is drawn from the pail and the motor immediately starts. The degree of pressure maintained is about one-half atmosphere, or 7½ pounds to the square inch. Leading from the pail cover or pulsator are two flexible tubes besides the one leading to the vacuum cock above the stanchions. At the end of each tube are 4 cups, which are fitted over the teats of the cow. The milk from the 2 cows is discharged into one pail (pl. 4, fig. 2). In operation the machine makes a low, clicking sound, which is caused by the motor. The vacuum pulsations run from 50 to 70 per minute and may be easily adjusted to the speed

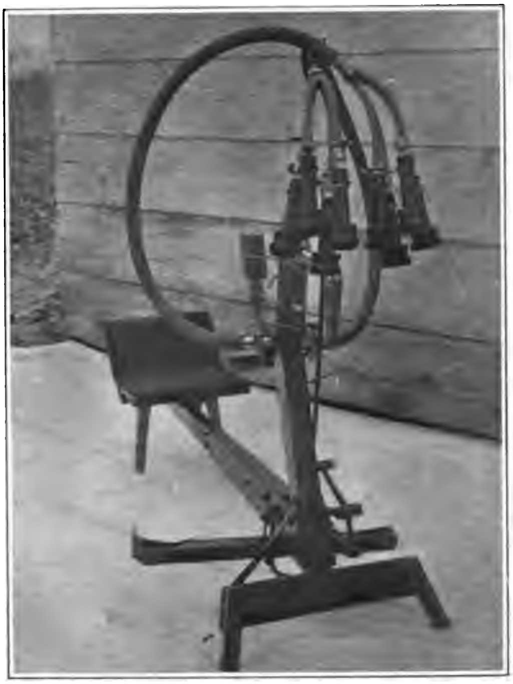

FIG. 1.—A FOOT-POWER COW MILKER WITH ATTACHMENTS.

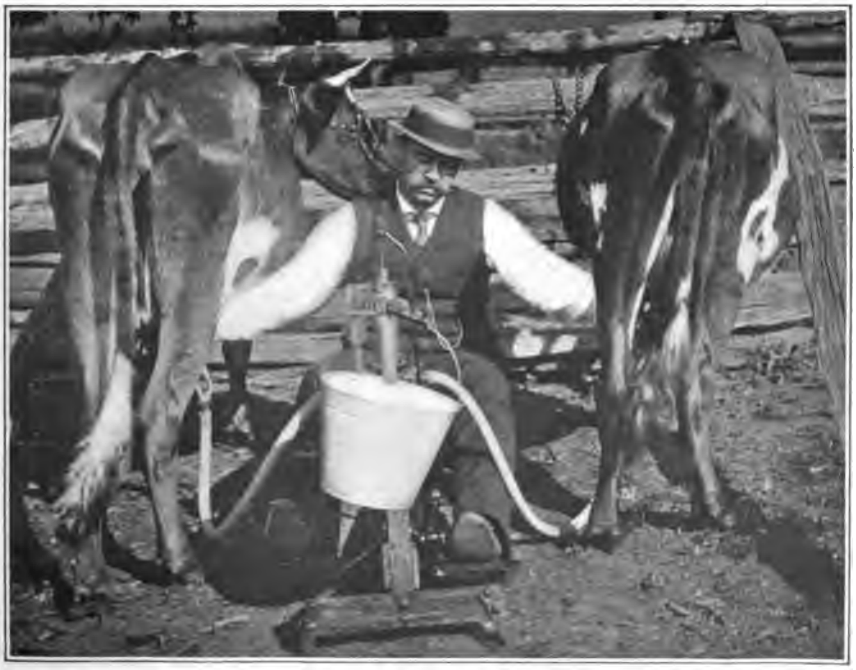

FIG. 2.—THE SAME MACHINE IN OPERATION.

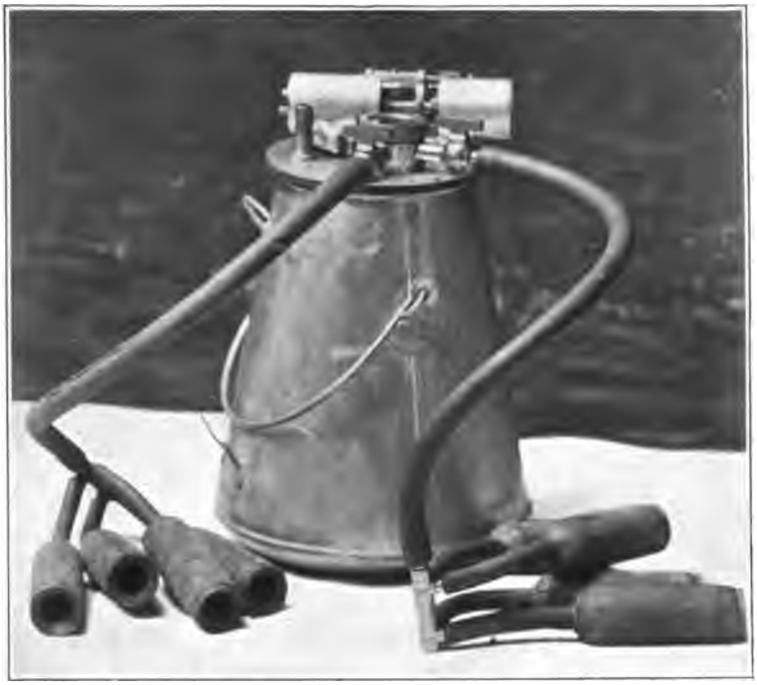

FIG. 1.—A POWER MILKER WITH ATTACHMENTS.

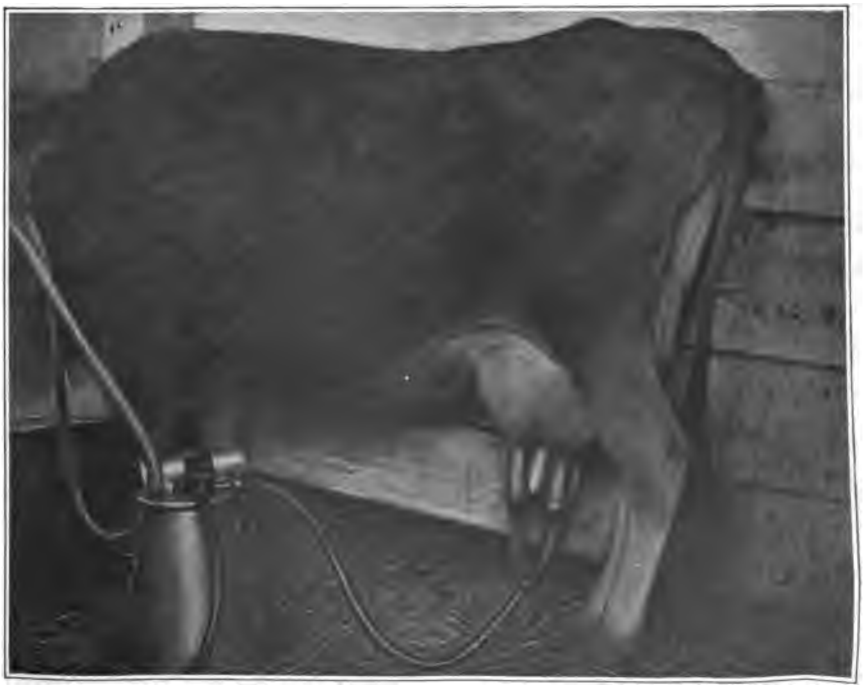

FIG. 2.—THE SAME MACHINE IN OPERATION.

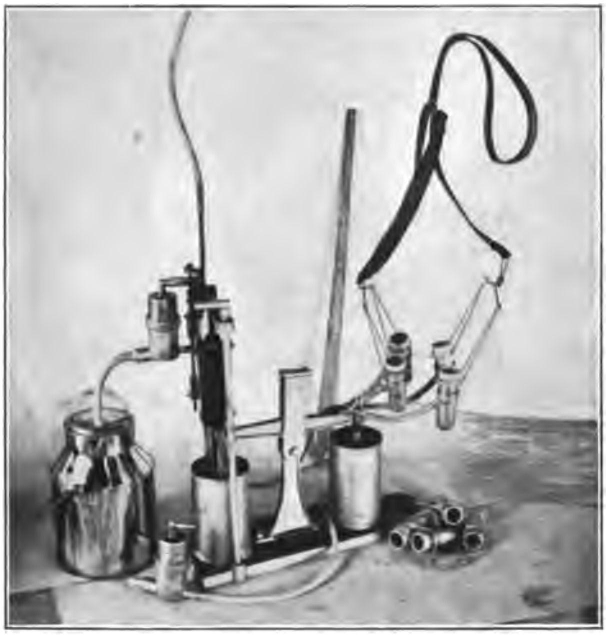

FIG. 1.—MILKING MACHINE DESIGNED FOR USE WITH EITHER HAND OR POWER.

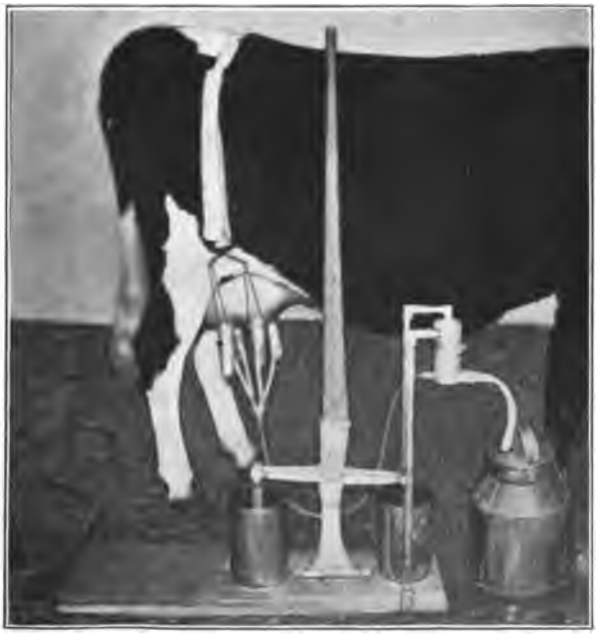

FIG. 2.—THE SAME MACHINE IN OPERATION.

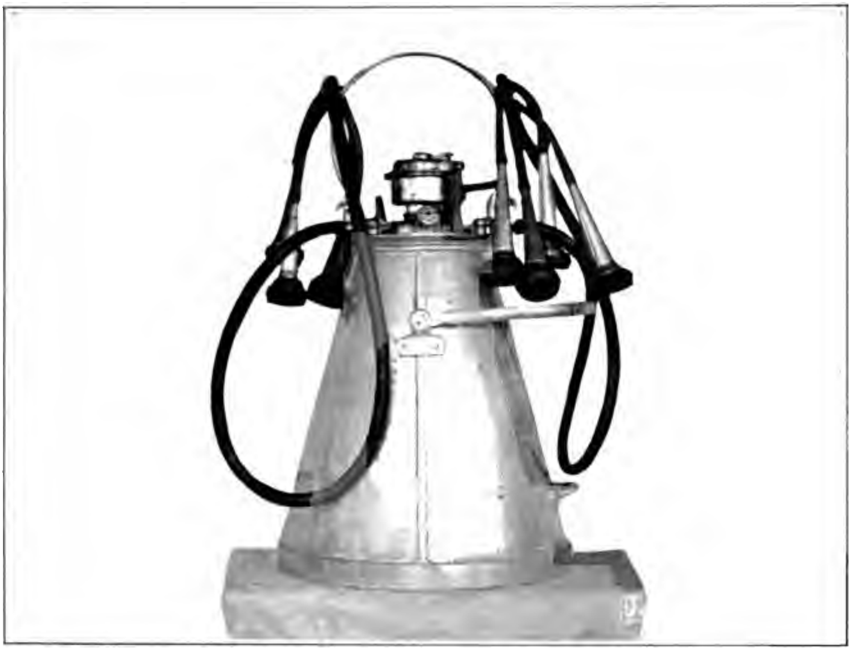

FIG. 1.—MILKING MACHINE USED IN EXPERIMENTS.

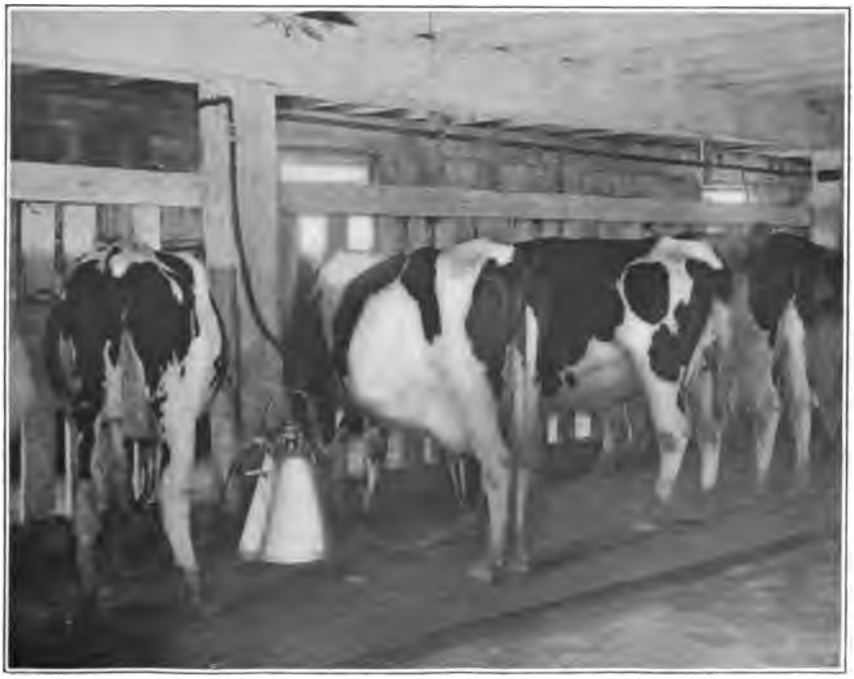

FIG. 2.—THE SAME MACHINE IN OPERATION.

required. The milk in passing from the cow to the pail goes thru a glass inspection tube, so that the operator may watch the flow. When the milk ceases to flow the suction is turned off and the action of the machine stops. Four different sizes of teat cups are provided, so that different sizes of teats may be fitted.

COST OF EQUIPMENT FOR MACHINE MILKING.

At the present time the equipment required to milk a herd of 40 cows with the machines and the cost of the same would be as follows:

1. An engine or some power with which to drive the machine. For milking up to 8 cows at a time, a 2-horsepower gasoline engine may be used, costing	$105.00
2. A vacuum pump, costing	75.00
3. A vacuum tank, like a tank that is used in connection with ranges or stoves in kitchens, costing	11.00
4. The piping with valves, etc., necessary in barn, depending upon extent of plant, number of cows, etc., costing for a 42-cow dairy about	25.00
5. Four milking machines, costing	300.00
Total	516.00

One machine milks 2 cows at a time, and it has been found practicable to allow one machine to every 10 or 12 cows when equipping the herd.

In a general way it may be said that the entire cost of installing a plant for herds of different sizes would be about as follows:

For a dairy of 30 cows, with 2 machines, milking 2 cows each or 4 cows at one time, cost per cow	$13.00
For a dairy of 40 cows, with 3 machines, milking 6 cows at one time, cost per cow	12.00
For a dairy of 60 cows, with 4 machines, milking 8 cows at one time, cost per cow	10.00
For a dairy of 75 cows, with 5 machines, milking 10 cows at one time, cost per cow	8.50
For dairy of 100 cows, with 8 machines, milking 16 cows at one time, requiring about a 4-horsepower engine and a larger pump, cost per cow	10.00

One good careful man or woman can operate 4 machines milking 8 cows simultaneously, and an additional hand can not only carry away the milk, but assist in manipulating the cows' udders. The operating expense of the machines is comparatively small.

DIFFERENT KINDS OF POWER WHICH MAY BE UTILIZED.

The kind of power employed to operate cow milkers is not important provided it is uniform and can be depended upon.

Dairymen well know that, in the case of hand milking, if they were to stop for a time when a cow was partially milked and then begin again and finish milking, the chances are that there would not only be a decrease in yield, but the milk would be of poorer quality. A similar effect is produced in the case of machine milking. If the engine, or whatever power is employed to work the pumps, stops for any cause during the milking, a marked decrease in the yield of milk results.

Gasoline engines.—These are most commonly employed for power at the present time.

Electric motors.—Some farmers located near cities find electricity the most convenient power. This has worked successfully on two farms at least. In one case a trolley line passes near the barn and a wire is attached to the main trolley wire and connected with a 1-horsepower electric motor inside of the building. As electric roads are now being rapidly built thru country districts it is quite possible that this may prove a popular method of securing power to operate cow milkers.

Steam power.—Steam engines are employed on some farms, and they will be found to work satisfactorily in supplying power to operate the milking machines. Where steam is used on farms for other purposes it can be made to run the milkers with but little extra expense.

EXPERIMENTS IN WHICH HAND AND MACHINE MILKING WERE COMPARED.

Two experiments were conducted by the Dairy Division in which tests were made to determine the time required to milk by hand and by machine. The yield of milk, its chemical composition, and other points that were considered of interest to dairymen, were also studied.

EXPERIMENT NO. 1.

From a herd of 13 cows, 8 were selected for this test, the others being nearly dry. The animals were Jerseys and Holsteins, all 5 years old or over. This herd had been milked with the machines for over three years.

The cows were divided into two lots of 4 each in such a way that each lot gave practically the same amount of milk. The test continued for thirty days and was divided into three periods of ten days each. At the beginning of the first period, Lot I was milked by hand and Lot II by machine. At the end of each period of ten days the methods of milking the two lots of cows were reversed so that the results from milking by the two methods could be compared. The milking was all performed by one man.

TABLE 1.—*Machine milking: Time required to milk, and yield of milk and strippings.*

The cows and date.	Morning.				Evening.				Total for day.			
	Time required.	Yield of milk.			Time required.	Yield of milk.			Time required.	Yield of milk.		
		Machine milk.	Strippings.	Total.		Machine milk.	Strippings.	Total.		Machine milk.	Strippings.	Total yield.
LOT II, 4 COWS.												
	Min.	*Lbs.*	*Lbs.*	*Lbs.*	*Min.*	*Lbs.*	*Lbs.*	*Lbs.*	*Min.*	*Lbs.*	*Lbs.*	*Lbs.*
July 21	14.00	30.50	1.25	31.75	13.00	25.00	1.50	26.50	27.00	55.50	2.75	58.25
July 22	14.00	36.50	.75	37.25	12.00	34.75	.80	35.55	26.00	71.25	1.55	72.80
July 23	14.00	31.50	.50	32.00	13.00	28.00	[a] 8.10	36.10	27.00	59.50	8.60	68.10
July 24	13.50	36.50	.75	37.25	15.00	36.50	.50	37.00	28.50	73.00	1.25	74.25
July 25	14.50	35.00	.50	35.50	11.50	35.00	.50	35.50	26.00	70.00	1.00	71.00
July 26	13.00	36.50	.50	37.00	12.50	34.50	.50	35.00	25.50	71.00	1.00	72.00
July 27	12.50	34.00	.25	34.25	12.00	32.00	.60	32.60	24.50	66.00	.85	66.85
July 28	13.00	30.50	[b] 5.25	35.75	14.00	34.80	.40	35.20	27.00	65.30	5.65	70.95
July 29	13.00	38.00	.75	38.75	13.50	32.00	.40	32.40	26.50	70.00	1.15	71.15
July 30	12.00	37.50	.50	38.00	12.00	29.25	.90	30.15	24.00	66.75	1.40	68.15
Total	133.50	346.50	11.00	357.50	128.50	321.80	14.20	336.00	262.00	668.30	25.20	693.50
Daily average	13.35	34.65	1.10	35.75	12.85	32.18	1.42	33.60	26.20	66.83	2.52	69.35
LOT I, 4 COWS.												
July 31	13.00	30.00	.75	30.75	12.50	27.30	1.80	29.10	25.50	57.30	2.55	59.85
August 1	13.50	34.50	.75	35.25	14.00	27.30	.50	27.80	27.50	61.80	1.25	63.05
August 2	13.00	34.00	.50	34.50	13.00	27.00	.50	27.50	26.00	61.00	1.00	62.00
August 3	12.50	36.00	.50	36.50	13.00	24.00	1.00	25.00	25.50	60.00	1.50	61.50
August 4	12.00	32.00	.75	32.75	13.00	24.50	.75	25.25	25.00	56.50	1.50	58.00
August 5	13.00	34.50	.75	35.25	15.00	26.25	1.00	27.25	28.00	60.75	1.75	62.50
August 6	12.50	32.50	.75	33.25	14.00	25.50	.30	25.80	26.50	58.00	1.05	59.05
August 7	13.00	33.00	.50	33.50	14.00	27.25	.75	28.00	27.00	60.25	1.25	61.50
August 8	12.50	33.50	.50	34.00	15.50	27.75	.75	28.50	28.00	61.25	1.25	62.50
August 9	13.00	34.25	.50	34.75	13.00	34.00	.40	34.40	26.00	68.25	.90	69.15
Total	128.00	334.25	6.25	340.50	137.00	270.85	7.75	278.60	265.00	605.10	14.00	619.10
Daily average	12.80	33.42	.62	34.05	13.70	27.08	.77	27.86	26.50	60.51	1.40	61.91
LOT II, 4 COWS.												
August 10	11.00	36.00	.50	36.50	11.25	30.10	1.00	31.10	22.25	66.10	1.50	67.60
August 11	12.00	36.00	.25	36.25	12.00	32.00	.25	32.25	24.00	68.00	.50	68 50
August 12	13.50	33.00	.50	33.50	15.00	[c] 27.75	5.25	33.00	28.50	60.75	5.75	66.50
August 13	12.50	34.00	.25	34.25	13.00	[d] 31.00	3.75	34.75	25.50	65.00	4.00	69.00
August 14	13.00	40.00	.25	40.25	16.00	26.00	1.50	27.50	29.00	66.00	1.75	67.75
August 15	14.00	39.50	.25	39.75	16.50	33.50	.25	33.75	30.50	73.00	.50	73.50
August 16	13.00	35.50	.50	36.00	13.50	31.00	.70	31.70	26.50	66.50	1.20	67.70
August 17	12.50	34.50	.25	34.75	14.00	36.50	.25	36.75	26.50	71.00	.50	71.50
August 18	13.50	33.00	.25	33.25	14.50	40.00	.50	40.50	28.00	73.00	.75	73.75
August 19	14.00	40.00	.50	40.50	16.00	[e] 37.50	5.75	43.25	30.00	77.50	6.25	83.75
Total	129.00	361.50	3.50	365.00	141.75	325.35	19.20	344.55	270.75	686.85	22.70	709.50
Daily average	12.90	36.15	.35	36.50	14.17	32.53	1.92	34.45	27.07	68.68	2.27	70.95
Grand total	390.50	1,042.25	20.75	1,063.00	407.25	918.00	41.15	959.15	797.75	1,960.25	61.90	2,022.10
General average	13.02	34.74	.69	35.43	13.57	30.60	1.37	31.97	26.59	65.34	2.06	67.40

[a] One cow held up milk (7.5 pounds) on account of stranger being in barn.
[b] One cow held up milk (5 pounds) on account of stranger being in barn.
[c] Black cow held up milk and gave 4.75 pounds by hand.
[d] Black cow held up milk and gave 3 pounds by hand (stranger in barn).
[e] Black cow held up milk and gave 5 pounds by hand.

TABLE 2.—*Hand milking: Time required to milk, and yield of milk and strippings.*

The cows and date.	Morning.				Evening.				Total for day.			
	Time required.	Yield of milk.			Time required.	Yield of milk.			Time required.	Yield of milk.		
		First yield.	Strippings.	Total.		First yield.	Strippings.	Total.		First yield.	Strippings.	Total.
LOT I, 4 COWS.	*Min.*	*Lbs.*	*Lbs.*	*Lbs.*	*Min.*	*Lbs.*	*Lbs.*	*Lbs.*	*Min.*	*Lbs.*	*Lbs.*	*Lbs.*
July 21	21.00	31.00	1.50	32.50	20.00	32.20	1.80	34.00	41.000	63.20	3.300	66.500
July 22	22.00	36.00	1.50	37.50	16.00	29.20	1.00	30.20	38.000	65.20	2.500	67.700
July 23	21.50	34.00	1.00	35.00	17.00	28.20	1.70	29.90	38.500	62.20	2.700	64.900
July 24	21.00	32.00	1.25	33.25	16.00	33.00	1.25	34.25	37.000	65.00	2.500	67.500
July 25	24.50	35.00	1.25	36.25	20.50	29.75	1.25	31.00	45.000	64.75	2.500	67.250
July 26	19.50	35.50	1.00	36.50	17.00	34.50	1.50	36.00	36.500	70.00	2.500	72.500
July 27	18.50	32.00	1.25	33.25	19.00	32.00	1.00	33.00	37.500	64.00	2.250	66.250
July 28	24.00	35.00	1.00	36.00	17.25	33.00	1.00	34.00	41.250	68.00	2.000	70.000
July 29	20.00	35.00	1.50	36.50	18.50	33.80	1.10	34.90	38.500	68.80	2.600	71.400
July 30	21 00	32.50	1.75	34.25	16.00	30.00	1.90	31.90	37.000	62.50	3.650	66.150
Total	213.00	338.00	13.00	351.00	177.25	315.65	13.50	329.15	390.250	653.65	26.500	680.150
Daily average	21.30	33.80	1.30	35.10	17.72	31.56	1.35	32.91	39.025	65.36	2.650	68.015
LOT II, 4 COWS.												
July 31	26.00	37.00	.75	37.75	21.00	33.50	1.00	34.50	47.000	70.50	1.750	72.250
August 1	25.50	31.00	.75	31.75	20.00	31.60	.50	32.10	45.500	62.60	1.250	63.850
August 2	21.50	34.00	.75	34.75	19.00	33.00	.75	33.75	40.500	67.00	1.500	68.500
August 3	23.00	26.00	[a] 7.50	33.50	21.00	31.50	.90	32.40	44.000	57.50	[a] 8.400	65.900
August 4	23.50	31.00	.75	31.75	20.00	33.75	1.30	35.05	43.500	64.75	2.050	66.800
August 5	21.00	36.00	.75	36.75	22.50	32.00	1.25	33.25	43.500	68.00	2.000	70.000
August 6	24.00	31.50	.50	32.00	19.00	31.00	.50	31.50	43.000	62.50	1.000	63.500
August 7	22.00	35.00	.50	35.50	23.00	30.70	1.00	31.70	45.000	65.70	1.500	67.200
August 8	23.00	36.50	.50	37.00	21.50	34.20	.75	34.95	44.500	70.70	1.250	71.950
August 9	24.00	36.00	.50	36.50	19.00	33.40	.50	33.90	43.000	69.40	1.000	70.400
Total	233.50	334.00	13.25	347.25	206.00	324.65	8.45	333.10	439.500	658.65	21.700	680.350
Daily average	23.35	33.40	1.32	34.72	20.60	32.46	.84	33.31	43.950	65.86	2.170	68.035
LOT I, 4 COWS.												
August 10	21.00	28.00	1.00	29.00	16.00	25.75	2.00	27.75	37.000	53.75	3.000	56.750
August 11	24.00	31.00	.75	31.75	19.00	24.00	.80	24.80	43.000	55.00	1.550	56.550
August 12	21.00	28 50	.50	29.00	20.00	25.25	1.10	26.35	41.000	53.75	1.600	55.350
August 13	21.00	30.00	1.25	31.25	16.00	28.50	1.25	29.75	37.000	58.50	2.500	61.000
August 14	21.00	33.00	.25	33.25	19.00	26.25	1.00	27.25	40.000	59.25	1.250	60.500
August 15	20.00	32.00	.50	32.50	19.00	29.00	1.25	30.25	39.000	61.00	1.750	62.750
August 16	21.00	30.00	1.00	31.00	16.00	28.20	1.50	29.70	37.000	58.20	2.500	60.700
August 17	21.00	31.00	.75	31.75	18.00	29.00	1.25	30 25	39.000	60.00	2.000	62.000
August 18	20.00	31.00	.75	31.75	17.00	32.00	1.25	33.25	37.000	63.00	2.000	65.000
August 19	20.00	32.50	.75	33.25	18.00	31.50	1.25	32.75	38.000	64.00	2.000	66.000
Total	210.00	307.00	7.50	314.50	178.00	279.45	12.65	292.10	388.000	586.45	20.150	606.600
Daily average	21.00	30.70	.75	31.45	17.80	27.94	1.26	29.21	38.800	58.64	2.015	60.660
Grand total	656.50	979.00	33.75	1,012.75	561.25	919.75	34.60	954.35	1,217.750	1,898.75	68.350	1,967.100
General average	21.88	32.63	1.12	33.76	18.71	30.66	1.15	31.81	40.592	63.29	2.285	65.570

[a] One cow held up milk (7 pounds).

TIME REQUIRED FOR HAND AND MACHINE MILKING COMPARED.

Naturally one of the first questions asked when a dairyman is considering the installation of milking machines is: How much time will be saved by their use? In the experiment outlined above one man performed the milking, 1 cow milker being used in case of the lots milked by machinery. The machine was operated by a 1-horsepower electric motor. By referring to Tables 1 and 2 the reader can readily compare from day to day the time required to milk by the two methods. A glance at the general averages for thirty days shows that the average time required for one man to milk 4 cows with the machine was 13.02 minutes in the morning and 13.57 minutes in the evening, or a total of 26.59 minutes for the day. These figures include the time used in putting the machine in place and adjusting the teat cups, about one-fourth minute per cow being required to adjust them properly.

In considering the time, the amount of milk secured should also be considered. Other things being equal, cows producing a large amount of milk require the most time to perform the milking. In case of the 4 cows milked by hand it will be noted that it took an average of 21.88 minutes to milk them in the morning and 18.71 minutes in the evening, or a total of 40.59 minutes for the day. There was therefore a daily saving of 3.5 minutes per cow, or 14 minutes on 4 cows, thru the use of the machines.

It should be noted, however, that the time saved by the employment of machines was not the result of greater speed in milking, but in the operator's ability to milk 2 cows at once. Indeed, one man can look after 5 machines milking 10 cows at once, thus greatly increasing this saving of time. (See experiment No. 2.)

Speed of the machine.—As stated in the description, the pulsator can be adjusted so that the action will be fast or slow; 50 to 60 pulsations per minute is the rate usually recommended. The more rapid the pulsations the faster the machine will milk, up to a certain limit. The writer saw one cow, giving a good flow, milked absolutely clean with a machine in 2¼ minutes, the number of pulsations being 150 per minute. It is believed, however, that such rapid milking for any length of time has a bad influence upon the cow and after a time she might object to the machine and refuse to give down her milk.

Cows vary greatly individually, and it is the best plan to adjust the speed of the machine as closely as possible to the requirements of each cow. Cows with short teats can be milked with the machine set at greater speed than cows with large, long teats. The ordinary type of

Ayrshire cow, for example, can probably be milked by more rapid pulsations than the Holstein. Those acquainted with milking these two types of cows by hand know this to be true, and that in milking some cows the milk is ready to be drawn as fast as the milker can manipulate the teats.

While the machines were in operation the attendants massaged the udders of the cows and watched to see that they were milking freely. Care was taken to treat the animals as nearly as possible the same at each milking, so they would become accustomed to the machines.

YIELD OF MILK FROM HAND AND MACHINE MILKING COMPARED.

The yield of milk is perhaps the most important matter to the dairyman. Any method of milking that has a tendency to decrease the flow to any appreciable extent can hardly be considered practicable. This point was studied for a period of thirty days in this experiment with the milking machine. While a much longer period is necessary to settle this question, the results secured indicate at least what may be expected when the machines are properly handled. In this test the cows were carefully handled, care being taken to adjust the teat cups to suit the individuality of each cow, also to use cups of the same size on each cow every time.

As already stated, two lots of cows were selected that yielded practically the same amount of milk. Referring to Tables 1 and 2 it will be noted that the total yield of milk for 4 cows during thirty days was 1,898.75 pounds from hand milking[a] and 1,960.25 pounds from machine milking,[b] not including strippings—a difference of 61.5 pounds, or 3.24 per cent, in favor of the machine. A study of the yields of the different lots of cows for the different periods shows that Lot I, which started with hand milking, gradually decreased in milk flow when changed to the machines and also continued to decrease when changed again to hand milking. The yields of Lot II, which began with machine milking, show that these cows decreased when changed to hand milking and materially increased in yield when changed back again to machine milking.

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[a] One cow held up part of her milk one day (see Table 2).

[b] One cow held up a small quantity of milk a few times and was finished by hand (see Table 1).

The yields of Lots I and II for the three 10-day periods were as follows:

Yields from machine milking and hand milking compared.

	Pounds.
Lot I, 4 cows:	
First 10-day period, cows milked by hand	653.65
Second 10-day period, cows milked by machine	605.10
Third 10-day period, cows milked by hand	586.45
Lot II, 4 cows:	
First 10-day period, cows milked by machine	668.30
Second 10-day period, cows milked by hand	658.65
Third 10-day period, cows milked by machine	686.85

As the cows of Lot II increased in yield when changed back again to machine milking in the third period, when the natural tendency was to decrease owing to advance in lactation, it appears that the machine had a favorable influence upon the production of this lot of cows.

As Lot I was used during two periods of the hand-milking test and in only one period with the machine test, and Lot II the reverse, a fairer comparison may be drawn by averaging the yields where the same lot of cows was used twice in either test. The result of this method of comparison is shown in the tabulation which follows:

Comparison of the milk yield of the two lots of cows (not including strippings).

	Pounds.
Hand milking:	
Lot I, average of first and third periods	620.05
Lot II, second period	658.65
Total	1,278.70
Machine milking:	
Lot II, average of first and third periods	677.50
Lot I, second period	605.10
Total	1,282.60

It appears that the yield of milk, not including strippings, amounted to 1,278.7 pounds from hand milking and 1,282.6 from machine milking, a difference of 3.9 pounds also in favor of the machine. This further comparison will serve to strengthen the conclusion already given that when the machines are properly handled and carefully adjusted to suit the needs of individual cows the yield of milk compares favorably with that of hand milking.

THORONESS OF HAND AND MACHINE MILKING COMPARED.

A machine that does not milk cows clean, or at least as clean as the average farm hand does, is of but little service to dairymen. This point was tested as thoroly as possible in the experiment just described. The man who milked the cows by hand in this experiment was instructed to milk the cows as normally as possible and not to go to extremes in either direction. It is believed that a fair trial was made in this respect.

Referring to Table 1 it will be observed that the amount of strippings was fairly uniform from day to day in case of both hand and machine milking. A comparison of the amount of strippings from each lot of cows for the three 10-day periods is shown in the following tabulation:

Comparison of amount of strippings in hand and machine milking.

	Pounds.
Hand milking:	
Lot I, first period	26.50
Lot II, second period	21.70
Lot I, third period	20.15
Total	68.35
Machine milking:	
Lot II, first period	25.20
Lot I, second period	14.00
Lot II, third period	22.70
Total	61.90

The total strippings from hand milking for the 4 cows for thirty days is shown to be 68.35 pounds, and from machine milking 61.90 pounds, or 6.45 pounds less for the machine. The average strippings per cow for each milking was 4.55 ounces by hand and 4.12 ounces by machine.

The composition of the milk was not determined in this experiment.

EXPERIMENT NO. 2.

This experiment was conducted with 20 cows selected from a herd of 65. All kinds of cows were selected for the test, including hard and easy milkers, heifers, and mature animals, and fresh cows as well as those advanced in lactation; also including cows of nervous temperament which it was known did not take kindly to the machine. This was done in order that a thoro study might be made. The 20 cows were divided into two lots of 10 each, which we will designate as Lots I and II.

Lot I was milked by hand and Lot II by machine for a period of 10 days; the methods of milking were then reversed and Lot I was milked by machine and Lot II by hand, 2 days being allowed between the periods to make the change. Five machines were used to milk the 10 cows. These were handled by one man. One man also milked the 10 cows by hand the greater part of the time. Whenever more than one man was employed the results were put on the basis of one man; that is, if two men milked the 10 cows in 40 minutes this was considered equivalent to one man milking 80 minutes. This experiment differed from the one previously described in that more cows were included and less attempt was made to adjust the teat cups to the different cows or to use the same machine on the same pair of cows every time, this matter being left largely to the dairyman in charge. These points will be considered in studying the results.

TABLE 3.—*A comparison of the yield and composition of milk from hand and machine milking.*

HAND MILKING.

The cows and date.	Morning.						Evening.						For the day.					
	Time required.	Yield.			Composition.		Time required.	Yield.			Composition.		Time required.	Yield.			Composition.	
		First milk.	Strippings.	Total.	Fat.[a]	Solids not fat.[a]		First milk.	Strippings.	Total.	Fat.[a]	Solids not fat.[a]		First milk.	Strippings.	Total.	Fat.[a]	Solids not fat.[a]
LOT I, 10 COWS.	*Min.*	*Pounds.*	*Lbs.*	*Pounds.*	*Per ct.*	*Per ct.*	*Min.*	*Pounds.*	*Lbs.*	*Pounds.*	*Per ct.*	*Per ct.*	*Min.*	*Pounds.*	*Lbs.*	*Pounds.*	*Per ct.*	*Per ct.*
July 26	82.0	121.0	1.00	122.00	3.70	8.41	72.00	117.50	1.00	118.50	4.60	8.38	154.00	238.50	2.00	240.50	4.15	8.40
July 27	80.0	101.0	1.00	102.00	4.20	8.38	54.00	95.00	2.00	97.00	3.80	8.75	134.00	196.00	3.00	199.00	4.00	8.57
July 28	75.0	123.0	2.00	125.00	3.70	8.65	80.00	106.00	2.50	108.50	3.70	8.65	155.00	229.00	4.50	233.50	3.70	8.65
July 29	70.0	119.0	1.50	120.50	3.60	8.53	75.00	101.50	1.50	103.00	4.00	8.69	145.00	220.50	3.00	223.50	3.80	8.61
July 30	80.0	118.0	1.00	119.00	3.50	8.59	70.00	103.50	1.50	105.00	4.00	8.56	150.00	221.50	2.50	224.00	3.75	8.58
July 31	80.0	121.0	1.50	122.50	3.40	8.44	74.00	97.00	2.00	99.00	3.90	8.54	154.00	218.00	3.50	221.50	3.65	8.49
August 1	80.0	121.5	3.00	124.50	3.60	8.48	70.00	96.00	1.50	97.50	3.90	8.67	150.00	217.50	4.50	222.00	3.75	8.58
August 2	75.0	110.0	1.00	111.00	3.70	8.70	65.00	110.00	1.50	111.50	3.80	8.62	140.00	220.00	2.50	222.50	3.75	8.66
August 3	80.0	120.5	1.00	121.50	3.60	8.61	80.00	91.00	1.00	92.00	3.90	8.79	160.00	211.50	2.00	213.50	3.75	8.70
August 4	85.0	117.0	1.50	118.50	3.90	8.61	70.00	101.00	1.50	102.50	3.90	8.61	155.00	218.00	3.00	221.00	3.90	8.61
Total	787.0	1,172.0	14.50	1,186.50	......	......	710.00	1,018.50	16.00	1,034.50	......	......	1,497.00	2,190.50	30.50	2,221.00	......	......
Average	78.7	117.2	1.45	118.65	3.70	8.54	71.00	101.85	1.60	103.45	3.95	8.62	149.70	219.05	3.05	222.10	3.82	8.58
LOT II, 10 COWS.																		
August 7	85.0	116.0	1.00	117.00	3.40	8.37	80.00	112.00	1.50	113.50	3.80	8.37	165.00	228.00	2.50	230.50	3.60	8.37
August 8	80.0	123.0	1.50	124.50	3.40	8.46	80.00	101.00	2.00	103.00	3.60	8.48	160.00	224.00	3.50	227.50	3.50	8.47
August 9	90.0	125.0	4.00	129.00	3.50	8.41	80.00	106.50	1.50	108.00	3.40	8.34	170.00	231.50	5.50	237.00	3.45	8.38
August 10	90.0	116.0	1.00	117.00	3.70	8.55	80.00	107.00	4.50	111.50	3.80	8.32	170.00	223.00	5.50	228.50	3.75	8.44
August 11	80.0	107.0	2.00	109.00	3.70	8.57	75.00	107.00	2.00	109.00	4.40	8.49	155.00	214.00	4.00	218.00	4.05	8.53
August 12	90.0	121.0	1.00	122.00	3.70	8.47	75.00	99.00	1.50	100.50	3.60	8.48	165.00	220.00	2.50	222.50	3.65	8.48
August 13	80.0	111.0	1.50	112.50	3.80	8.47	85.00	91.00	1.00	92.00	3.90	8.42	165.00	202.00	2.50	204.50	3.85	8.45
August 14	88.0	111.0	2.00	113.00	3.90	8.51	70.00	100.00	1.50	101.50	3.80	8.57	158.00	211.00	3.50	214.50	3.85	8.54
August 15	75.0	107.0	1.00	108.00	3.40	8.54	73.00	97.00	3.50	100.50	3.20	8.43	148.00	204.00	4.50	208.50	3.45	8.49
August 16	76.0	119.0	2.00	121.00	4.00	8.41	70.00	104.00	1.50	105.50	4.00	8.46	146.00	223.00	3.50	226.50	4.00	8.44
Total	834.0	1,156.0	17.00	1,173.00	......	......	768.00	1,024.50	20.50	1,045.00	......	......	1,602.00	2,180.50	37.50	2,218.00	......	......
Average	83.4	115.6	1.70	117.30	3.65	8.48	76.80	102.45	2.05	104.50	3.75	8.44	160.20	218.05	3.75	221.80	3.72	8.46
Grand total	1,621.0	232.8	31.50	1,359.50	......	......	1,478.00	2,043.00	36.50	2,079.50	......	......	3,099.00	4,371.00	......	4,439.00	......	......
General average	81.0	116.4	1.57	117.97	3.67	8.51	73.90	102.15	1.82	103.97	3.85	8.53	154.90	218.55	3.40	221.95	3.77	8.52

MACHINE MILKING.

LOT II, 10 COWS.																		
July 26	23.0	97.0	1.50	98.50	3.30	8.45	20.00	101.50	1.50	103.00	3.50	8.46	43.00	198.50	3.00	201.50	3.40	8.46
July 27	12.0	93.0	3.50	96.50	3.00	8.49	b 27.00	94.00	2.00	96.00	4.00	8.53	39.00	187.00	5.50	192.50	3.50	8.51
July 28	22.0	110.0	2.50	112.50	3.40	8.82	17.00	106.00	2.50	108.50	3.90	8.29	39.00	216.00	5.00	221.00	3.65	8.56
July 29	20.0	116.0	2.50	118.50	3.10	8.43	20.00	90.00	1.50	91.50	3.40	8.57	40.00	206.00	4.00	210.00	3.25	8.50
July 30	20.0	100.0	6.00	106.00	3.60	8.88	15.00	c 101.00	1.50	102.50	3.50	8.59	35.00	201.00	7.50	208.50	3.55	8.74
July 31	15.0	119.5	1.50	121.00	3.10	8.51	d 13.00	87.00	3.50	90.50	2.70	8.54	28.00	206.50	5.00	211.50	2.90	8.53
August 1	17.0	115.0	2.00	117.00	2.70	8.39	17.00	90.00	2.50	92.50	3.40	8.59	34.00	205.00	4.50	209.50	3.05	8.49
August 2	15.0	e 130.0	2.00	132.00	3.20	8.73	13.50	c 106.00	3.50	109.50	3.50	8.53	28.50	236.00	5.50	241.50	3.35	8.63
August 3	22.0	e 110.5	1.50	112.00	3.20	8.50	17.00	e 87.00	1.50	88.50	4.00	8.71	39.00	197.50	3.00	200.50	3.60	8.61
August 4	20.0	e 130.0	2.00	132.00	3.20	8.50	20.00	108.00	2.00	110.00	3.80	8.57	40.00	238.00	4.00	242.00	3.50	8.54
Total	186.0	1,121.0	25.00	1,146.00	......	......	179.50	970.50	22.00	992.50	......	......	365.50	2,091.50	47.00	2,138.50	......	......
Average	18.6	112.1	2.50	114.60	3.20	8.57	17.95	97.05	2.20	99.25	3.60	8.54	36.55	209.15	4.70	213.85	3.37	8.55
LOT I, 10 COWS.																		
August 7	17.0	112.0	2.00	114.00	3.70	8.43	15.00	94.00	1.50	95.50	4.20	8.68	32.00	206.00	3.50	209.50	3.95	8.56
August 8	20.0	123.0	1.00	124.00	3.20	9.17	17.00	102.00	2.00	104.00	3.80	8.37	37.00	225.00	3.00	228.00	3.50	8.77
August 9	23.0	e 112.0	2.00	114.00	3.10	8.45	21.00	100.00	1.00	101.00	4.10	8.33	44.00	212.00	3.00	215.00	3.60	8.39
August 10	20.0	98.0	2.50	100.50	3.40	8.64	21.00	101.00	1.50	102.50	3.80	8.42	41.00	199.00	4.00	203.00	3.60	8.53
August 11	18.0	101.0	.50	101.50	3.70	8.70	14.00	102.00	2.50	104.50	3.80	8.67	32.00	203.00	3.00	206.00	3.75	8.69
August 12	16.0	85.0	1.50	86.50	3.70	8.45	17.00	87.50	1.50	89.00	3.50	8.81	33.00	172.50	3.00	175.50	3.60	8.63
August 13	22.0	e 98.0	1.50	99.50	3.50	8.83	12.00	81.00	1.00	82.00	4.00	8.43	34.00	179.00	2.50	181.50	3.75	8.63
August 14	25.0	100.0	6.00	106.00	3.50	8.63	22.00	84.50	2.00	86.50	3.20	8.83	47.00	184.50	8.00	192.50	3.35	8.73
August 15	20.0	102.0	1.50	103.50	3.30	8.64	17.00	92.00	1.00	93.00	3.80	8.42	37.00	194.00	2.50	196.50	3.55	8.53
August 16	21.0	103.0	1.20	104.20	3.20	8.70	18.00	93.00	4.50	97.50	3.50	8.21	39.00	196.00	5.70	201.70	3.35	8.46
Total	202.0	1,034.0	19.70	1,064.50	......	......	174.00	937.00	18.50	955.50	......	......	376.00	1,971.00	38.20	2,009.20	......	......
Average	20.2	103.4	1.97	106.45	3.50	8.66	17.40	93.70	1.85	95.55	3.90	8.52	37.60	197.10	3.82	200.92	3.60	8.59
Grand total	388.0	2,155.0	44.70	2,210.50	......	......	353.50	1,907.50	40.50	1,948.00	......	......	741.50	4,062.50	85.20	4,147.70	......	......
General average.	19.4	107.7	2.23	110.52	3.30	8.61	17.67	95.37	2.02	97.40	3.75	8.53	37.07	203.12	4.26	207.39	3.49	8.57

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[a] Average of percentages only.

[b] Cow had inflamed udder, requiring longer time to milk.

[c] Too small teat cups used on cow, causing her to hold up milk.

[d] One hundred and ten pulsations.

[e] One to 3 cows held up some of their milk and were finished by hand.

TIME REQUIRED FOR HAND AND MACHINE MILKING COMPARED.

The accompanying table shows the time required to milk the cows by the two methods during the different periods. In this experiment one man handled 5 machines with little difficulty. The man who milked the cows by hand was unusually thoro and took somewhat more time to do the work than the average milker.

It will be noted that the average time required for one man to milk the 10 cows with the machines for 20 days was 19.4 minutes in the morning and 17.67 minutes in the evening, or a total of 37.07 minutes for the day. The time stated includes the time required for putting the machines in place, adjusting the teat cups, and milking; the average time per cow being 1.94 minutes in the morning and 1.76 minutes in the evening, or a total of 3.7 minutes for the day.

In the case of the 10 cows milked by hand it will be noted that it required for the 20 days an average of 81 minutes in the morning and 73.9 minutes in the evening, or a total for the day of 154.9. The average time required per cow was therefore 8.1 minutes in the morning and 7.39 minutes in the evening, or a total of 15.49 minutes for the day. On the basis of these results the daily saving per cow thru the use of the machines amounted to 11.79 minutes, or 117.9 minutes for the 10 cows. Carrying the comparison still further, the saving for 60 cows (the number in this herd) would amount to 11.7 hours per day if one man performed all of the work, which of course would not be possible for a herd of this size, altho the writer has known one man to milk 60 cows with 6 machines on more than one occasion, the time required being about 2 hours. If, however, we include the time of the man who removed the milk and assisted in manipulating the cow's udders the saving of time is reduced to one-half, or 58.45 minutes per day for the 10 cows. These figures furnish sufficient proof for the statement that the machines are "time savers."

YIELD OF MILK FROM HAND AND MACHINE MILKING COMPARED.

As previously stated, but little attention was given to properly adjusting the machines by the attendants in this herd. Further than this, it was known that 1 or 2 of the cows selected did not take kindly to the machine, but it was desired to have the cows in the test represent the average of the herd. While a longer period than 20 days would have been desirable, the results indicate, at least, what may be expected under the conditions which prevailed during this test.

Referring to Table 3, it will be observed that the total yield of milk for 10 cows during the 20 days was 4,371 pounds from hand milking and 4,062.5 pounds from machine milking, not including strippings—a difference of 308.5 pounds, or 7.59 per cent, in favor of hand milking.

This result is not surprising when we consider the careless manner in which the machines were adjusted to the animals, and that this frequently resulted in the animals holding up their milk. Further, the fact should be noted that the hand milker in this instance was unusually careful, taking an average of 7.75 minutes to milk a cow at each milking.

The results tend to show that where milking machines are used carelessly they are a disadvantage to the dairyman from the standpoint of yield. The same holds true of other machinery on the farm; the more complex it is the more care and skill is required to secure the best results; but with skill and care the more machinery the better, up to a certain limit. This is brought out in experiment No. 1, where the milking machine held its own or showed an advantage at every point.

THORONESS OF HAND AND MACHINE MILKING COMPARED.

As previously stated, the man who did the milking in this experiment was an unusually thoro milker and consequently took more time for milking than the average milker. While possibly it would have been better to have employed an average milker in this experiment in order to make a fair comparison, the question as to whether the milking was thoro by both methods can be made clear. In a few instances 1 or 2 cows refused to give down part of their milk (probably owing to the teat cups not being of the proper size). This of course increased the amount classed as strippings. A comparison of the amount of strippings (including the amount held up by the machine-milked cows, as explained above) from each lot of cows for the 10-day periods is as follows:

Comparison of amount of strippings in experiment No. 2.

	Pounds.
Hand milking:	
Lot I, first period	30.50
Lot II, second period	37.50
Total	68.00
Machine milking:	
Lot II, first period	47.00
Lot I, second period	38.20
Total	85.20

The total strippings from the hand and machine milking for the 10 cows during 20 days is thus shown to be 68 pounds and 85.2 pounds, respectively. On this basis the average strippings per cow at each milking amounted to 2.7 ounces by hand and 3.4 ounces by machine. In either case the results show unusually clean milking.

QUALITY OF THE MILK.

Percentage of butter fat in milk.—A sample of the mixt milk was taken at every milking in experiment No. 2 and tested for percentage of fat by the Babcock method. The results of the daily tests are shown in Table 3 and the average for the different periods has been brought together for ready comparison in the following tabulation:

Percentage of butter fat in milk drawn by hand and by machine.

	Per cent.
Hand milking:	
Lot I, first period (10 days)	3.82
Lot II, second period (10 days)	3.72
Average	3.77
Machine milking:	
Lot II, first period (10 days)	3.37
Lot I, second period (10 days)	3.60
Average	3.49

It will be noted that the average percentage of fat in the milk drawn by hand for the twenty days was 3.77 and in the milk drawn by machine 3.49, a considerable difference in favor of the hand milking.

A calculation of the yield of butter from the 4,439 pounds of milk resulting from the hand milking (Table 3), with an average of 3.77 per cent of butter fat, gives about 195.25 pounds of butter. The same amount of milk with an average of 3.49 per cent of butter fat would yield about 180.75 pounds of butter, or 14.50 pounds less owing to the lower percentage of butter fat presumably resulting from the use of the machines. If the yield of butter be calculated from the 4,147.7 pounds of milk resulting from machine milking, with an average of 3.49 per cent of fat, it will be found to be about 169 pounds. Thus, if we allow for both the lower yield and the lower percentage of fat in the milk in this experiment, the balance against the machine with 10 cows for twenty days would be about 26 pounds of butter, or about 13 per cent. This appears to be quite an unfavorable showing for the machine, but further experiments are necessary to determine the effect of machine milking on the composition of milk, as it is always unsafe to base general conclusions on the results of a single experiment.

Percentage of solids not fat.[a]—The percentage of solids not fat was determined by means of the lactometer. Tests were made from samples taken both morning and evening. The detailed results are given in Table 3, but in order to make a more ready comparison only the averages are presented here.

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[a] These tests were made by Prof. W. A. Stocking.

Percentage of solids not fat in milk drawn by hand and by machine.

	Per cent.
Hand milking:	
Lot I, first period (10 days)	8.58
Lot II, second period (10 days)	8.46
Average	8.52
Machine milking:	
Lot II, first period (10 days)	8.55
Lot I, second period (10 days)	8.59
Average	8.57

The final averages shown above for the solids not fat are very close, being 8.52 and 8.57, respectively, for the hand and machine milk. While further study should be made on this point, the figures secured indicate that there is practically no difference in the amount of solids not fat between machine and hand drawn milk.

GENERAL CONSIDERATIONS.

EFFECTS OF MILKING MACHINES UPON THE COWS.

First time machine is used.—It is of interest to note how the milking machine affects a herd of cows the first time it is used. The writer was present in a barn of about 40 cows on one occasion the first time the machines were put in operation. Some of the animals were a little restless at first, owing to the sight of the machines and the clicking of the pulsators, but soon they became quiet and reconciled to their action. One feature which is perhaps a little surprising is that heifers took to the machines as readily as the older cows. Only one cow in the herd in question made any disturbance at all while the machines were being attached, and this was due principally to attaching the machine on the opposite side from that on which the cow had been accustomed to be milked by hand. This cow, however, soon became quiet. The majority of the cows appeared to like the machines, and stood quietly chewing their cuds without manifesting any discomfort. A careful examination was made of the cows' teats and udders in several dairies where the machines had been in operation for several months (in one case over three years), and no ill effects were discovered.

Nervous cows.—On two or three occasions it was observed that when strangers came into the barn during milking time a cow would appear frightened and refuse to give down all of her milk. This occurred with cows being milked either by hand or by machine. When the machines are properly adjusted, cows of a nervous disposition do not seem to resent the method.

Kicking cows.—The writer, after visiting from time to time several herds where the machines were in operation and observing the machines working continuously in one large herd for a month, failed to find any cows that could not be milked because of the "kicking" habit. Reports from dairymen who have had experience with the machines confirm this statement. It is therefore believed that the machines give less trouble with such animals than the hand method.

Hard milkers.—The hard milker and the cow with small teats are milked much more easily and quickly by machine than by hand, and with the installation of milking machines there will be less reason for disposing of such cows. With the hard milker it is simply a question of keeping the machine at work a little longer, and with the small-teated cow care must be taken to have the teat cups of the proper size, and the pulsations may also be a little more rapid.

Heifers.—The results already secured indicate that the milking machine is particularly well adapted to heifers. Since milking is an artificial process, heifers that have never been milked by hand, as a rule, become much more quickly reconciled to the machine than older animals which have been milked for years by hand. The latter often have their udders more or less distorted and drawn out of shape by the hand method and do not as readily adapt themselves to the machine method.

Cows that refuse to be milked with the machine.—Occasionally a cow for some reason refuses to give down either a part or all of her milk. This does not seem to be due to nervous temperament. It is possible that the change from hand to machine milking is too radical for these particular individuals and that they need more time to become accustomed to it. Such cows do not make any objection to the machines being attached to them, but simply stand quietly and allow the machines to pump away without yielding any milk. As the cows that give this trouble are very few in number, dairymen who install machines usually dispose of them. Frequently, however, cows that refuse to give their milk at first milk freely after a short time.

ADJUSTING THE MACHINES TO THE COWS.

Great care should be taken to see that the proper-sized teat cups are used for each cow. Cows with large teats require larger and longer cups. If the cup is too small the teat will wedge into it and not milk; if too large it will not milk properly nor stay on so well. It has been found that a cow requires slightly larger cups when fresh than she does when advanced in the period of lactation. When fresh the udder is distended and a very small portion of it projects into the teat cup. Later, the udder as well as the teats are softer and more limp and project farther into the cup. During the tests made with the machines a cow occasionally refused to give down her milk simply because the teat cups used were not of the proper size; after new ones were adjusted the cow milked out clean. This emphasizes the importance of having cows always take the same places in the barn and using teat cups of the same size at each milking. Cows will then become accustomed to the cups and will milk clean.

PRACTICABILITY OF USING MACHINES FOR LARGE AND SMALL HERDS.

Naturally the large dairyman will be the first to adopt the cow milker for the reason that his equipment will cost him less per cow than the small dairyman. Again, the large dairyman has more at stake and has to depend entirely upon the hired men to do the work. If they fail him the work falls upon himself or perhaps upon a very limited number of helpers. With the installation of the milking machine the large dairyman is much more independent, and if necessary could milk a herd of 50 cows without assistance. This would be next to impossible without it. However, there seems to be no good reason why a dairyman with a herd of even 10 or 12 cows could not use a machine with profit. The power required could be secured at small cost, and the time saved could be used to advantage in working the team longer on the farm or in other ways.

HOW THE GENERAL INTRODUCTION OF THE MILKING MACHINE WOULD AFFECT THE DAIRY INDUSTRY.

The scarcity of milkers and the unreliability of many of them has had a tendency to keep many men from going into dairy farming. Some dairymen who have been in the business have been obliged to give it up for this reason. Great interest therefore centers around the milking machine, especially where the above difficulty exists. With the introduction of the milking machine only about one-half the labor will be required to milk the cows, and it is believed that the labor employed will be of a higher class than heretofore and will also command higher wages. It is believed also that the advent of the milking machine will have a tendency among farmers who now have small dairies to enlarge their plants and to make dairying their chief business. The trouble has been in the past that too many farmers have made dairying secondary to other work, and when anything had to be neglected it was always the dairy. For this reason the profits from their dairies have been small. Where the milking machines have been introduced they have influenced dairymen to clean up their barns and take more pride in their work. This naturally will result in the production of cleaner milk and perhaps in some cases in better prices. Any new apparatus which has a tendency to improve dairy conditions should be welcomed by the industry.

SUGGESTIONS FOR IMPROVEMENTS.

Improvements will doubtless be made in this milking machine from time to time. At present two cows are milked in the same can, thus making it impossible to secure the weight of the milk from single cows. A great deal of importance is now being placed upon records of individual cows in order to determine whether they are giving a profit to their owner, and this is a movement in the right direction. If individual cans were provided for the cows, accurate records could be secured for each animal. The difficulty of making machines for individual cows is not insurmountable; in fact a few machines have already been built which keep the milk from the two cows separate.

Another improvement that it is believed would add much to the practicability of the machine is a teat cup that could readily be adjusted to teats of any size, or shape. At present cups of different sizes are used to meet the requirements of different animals. Some improvements in this direction are already in progress.

OBJECTIONS AND DIFFICULTIES.

As attention has been called to the advantages to be secured by the use of milking machines, it is proper to point out some of the objections and possible difficulties.

Initial outlay.—The considerable outlay in the beginning will prove a serious obstacle to dairymen of limited means; and, even with those who are financially able to make the investment, it will have a tendency to deter dairymen from the installation of machines until their practical utility has been fully demonstrated.

Careless or improper use.—As already suggested, the machines may be improperly or carelessly used. The operator may fail to select and use teat cups which fit, etc.

Life of the machine.—The machine or some of its parts may prove short-lived, thus causing expense and trouble.

Troubles with the engine.—In case a steam, gasoline, or gas engine is used (as will usually be the case), it will be necessary to employ some one who has sufficient knowledge and experience to run the engine. To do otherwise is to court disaster. Even with such a man, trouble with the engine may occur any day. Besides, the use of an engine in or near the barn involves danger of fire, and makes extra precautions necessary to avoid invalidation of fire insurance on the building.

Stoppages and breakdowns.—In the operation of any kind of machinery there is always danger that some part will break down or get out of order. With most machinery this is not a very serious obstacle, since the work to be done can await repairs. But milking is work that can not be postponed. Attention has been called to the independence of the dairyman who milks with machines as compared with the one who must depend on hired help to milk his cows by hand, inasmuch as the latter may find himself short of help at any time. The situation, however, is reversed when the engine or some other vital part of the equipment breaks down or gets seriously out of order. If this occurs at or during milking time (as is likely), the dairyman who has installed machines, with 40 or more cows to milk, will find it very difficult, if not impossible, to bring in milkers enough to do the work by hand; and the difficulty will be aggravated if he loses time in unsuccessful attempts to repair the machinery.

Cleaning and care of the machines.—As this matter properly belongs in the second part of this bulletin, it need only be mentioned here as one of the difficulties which will demand the most serious attention of the dairyman who may attempt the use of milking machines.

NEED FOR FURTHER INVESTIGATIONS.

It is believed that the present investigations have yielded much valuable data, but they have been too limited in scope and time to justify final positive conclusions, even if the results had been uniform in their indications. The fact that they were not entirely so emphasizes the need for further investigation.

Unsettled questions.—Just when and how milk is formed in the udder of the cow, and the influences favorable or unfavorable to its formation, are subjects which are not well understood. Whether a purely mechanical device attached to the cow's teats to withdraw the milk from her udder will stimulate and favorably influence the formation of milk as well as the hands of the living milker must be considered an unsettled question.

Yield of milk.—In the matter of yield, experiment No. 1 gave a result slightly in favor of the machine, while experiment No. 2 gave a result slightly unfavorable to it. But it must be conceded that these experiments were too limited to settle definitely the question of yield even for short periods, while the effect of the continuous use of machines during full lactation periods was, of course, not tested at all.

Composition of the milk.—The composition of the milk is a matter of as great importance as the yield, and the effect on composition, of the means and methods employed in milking, is a part of the unsettled problem enunciated above. In experiment No. 1 the composition of the milk was, unfortunately, not tested. In experiment No. 2 the milk drawn by hand showed a considerably higher percentage of butter fat than that drawn by machines. It would of course be premature to conclude from this single test that machine milking will have an unfavorable influence on composition of the milk, but the need for more exhaustive investigation is certainly apparent.

Consideration such as the foregoing will naturally cause practical dairymen to hesitate regarding the installation of milking machinery until further careful experimentation yields fuller information and justifies more positive conclusions.

RESULTS OF EXPERIENCE WITH MILKING MACHINES AS REPORTED BY DAIRYMEN.

In order to have an expression of opinion direct from the dairymen regarding the use of cow milkers, a number of questions were sent to all dairymen who were known to have used machines for any length of time.

All of the 11 dairymen who reported said the effect of the machines upon the teats and udder of the cows was favorable; likewise the effect upon hard milkers, nervous cows, and kickers.

Nine reported that heifers adapted themselves readily to the machines; 1 stated that heifers took to them more readily than old cows, and 1 reported no experience. Of the five instances where 1 man handled 2 machines, the average number of cows milked was 23 and the average time required to milk them was 47 minutes, or practically 2 minutes per cow. In the one instance where 1 man handled 3 machines he milked 30 cows in 60 minutes. In the case where 2 men handled 4 machines 27 cows were milked in 40 minutes. In the two instances where 2 men handled 5 machines they milked an average of 52½ cows in 68½ minutes. Where 2 men handled 6 machines the time required was 1 minute per cow. Again where 3 men and boys were just learning they milked 30 cows in 55 to 75 minutes.

Some of the advantages of the machines reported were less help, less time required, and more and cleaner milk. One dairyman reported the cost to be a disadvantage, and another considered it a disadvantage to run the gasoline engine in winter.

From 1 to 4 cows in the different herds were reported as refusing to give their milk with the machine. Some reported having this trouble at first but said that later it disappeared.

Eight dairymen out of 11 did not think it necessary to strip the cows after using the machine, 2 thought it advantageous, and 1 simply stated that there was very little milk left after using the machines.

Six dairymen stated that they found little difference in the amount of milk produced, whether the cows were milked by hand or machine; 4 thought the machines increased the flow, and 1 stated that the effect of the machines on production was good.

All of the dairymen reported machine milking to be superior to hand milking.

ADDENDUM.

Since the experiments described in this bulletin were conducted (August, 1905), the condition of the milking-machine industry has greatly changed. Certain improvements have been made. For example, the milk ports thru the pulsator have been enlarged; a filter between the pail and the stanchion has been introduced, greatly reducing the number of bacteria; and the surging of the milk in the tubes has been prevented. The sanitary features have also been improved. Over a thousand of the machines are now reported to be in use.

II.—BACTERIOLOGICAL STUDIES OF A MILKING MACHINE.

By W. A. Stocking, Jr., M. S. A.,
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PRODUCTION OF SANITARY MILK.

At the present time there is a greater demand for milk which has been produced and cared for under such sanitary conditions as to insure its reaching the consumer in a pure, wholesome condition than for any other dairy product. In order to supply this demand the milk must reach the consumer in as nearly as possible the condition in which it leaves the udder of the healthy cow. One of the great difficulties in the way of supplying consumers with such a grade of milk lies in the dirt and the bacterial contamination which it receives before leaving the farm. As our knowledge of the laws of sanitation and the importance of pure foods becomes more widely disseminated among milk users this demand for a cleaner and more wholesome grade of milk will steadily increase. One of the greatest problems for the milk producer to solve is, therefore, that of producing such a grade of milk. A few dairymen are now producing a high grade of milk under such sanitary conditions as reduce the dirt and bacterial contamination to a minimum. For this purpose covered milk pails are commonly used, and great care is taken that the cows, the stables, the utensils, and the milkers shall be kept as clean as possible. All this extra care and labor is costly, and the milk thus produced demands a price considerably above the ordinary market price. This grade of milk fills a limited demand for those who can afford to pay the considerably increased price, but the problem of the milk supply for our cities will not be solved until the general consumer can be supplied with pure, clean milk at a reasonable price.

While the exercise of this increased care and the use of the covered pail reduce the chance for external contamination very materially and greatly improve the quality of the milk, the ideal condition would be to draw the milk directly from the udder into a closed receptacle without any chance for external contamination. This idea has stimulated in no small degree the inventors of the various styles of milking machines which have been developed during the last few years.

While it has been possible with some of these machines to draw the milk thru closed tubes directly from the udder into a closed receptacle, none of them have thus far proved satisfactory in all respects. If a machine could be devised which would be satisfactory in its effect upon the cow and upon the yield of milk, and which can be cleaned with reasonable ease and at the same time all contact of the milk with the surroundings could be prevented, it would go far toward solving the problem of providing the public with clean, wholesome milk.

EXPERIMENTS DESIGNED TO TEST THE SANITARY CHARACTER OF MACHINE-DRAWN MILK.

In planning this experimental work the writer had three main objects in view: (1) To determine the germ content of machine-drawn milk in comparison with that drawn by hand; (2) to test the efficiency of the machine in reducing the germ content to the minimum, and thus to determine its value in the production of "sanitary" milk; and (3) to determine the amount of care necessary to keep the machines in a sterile condition.

Two farms were chosen for this work; one, a dairy where the sanitary conditions were about the average existing in dairy barns, and the other representing a higher class of dairy barns in which sanitary milk might be produced.

EXPERIMENTS AT FARM NO. 1.

The first barn selected for this series of experiments is believed to represent about the average sanitary conditions of dairy barns in the principal dairy sections of the country. As may be seen by referring to the illustration (pl. 4, fig. 2), the barn itself was in good condition. The feeding alley, mangers, and drop were all of cement, while the stable floor on which the cows stood was of plank. The stable had been whitewashed and was fairly clean, but no special care was exercised each day in cleaning either the stable or cows nor in handling the milk.

METHOD OF EXPERIMENT.

In order to eliminate the difference due to the germ content of individual udders and give greater practical value to the work, two lots of 10 cows each were used in this series of tests. One of these lots was milked by machine and the other by hand. Samples from the mixt milk of each lot of 10 cows were taken as soon as the milking was completed and were carried to the laboratory. Owing to circumstances it was impossible to plate samples at night. The evening samples were therefore placed on ice until the following morning. The morning samples were also placed on ice as soon as obtained and plated after they were thoroly chilled. It is of course possible that there may have been some slight increase in numbers in the samples of evening's milk before they were plated. It is probable, however, that there was normally no appreciable increase but rather a decrease in total numbers, due to the natural dropping out of certain species in accordance with the so-called "germicidal property" of milk. In either case the value of the results is not affected, since the hand-drawn and machine-drawn samples were treated alike in every case, and it is the comparison of these which is of value. In order to determine the numbers of bacteria existing in the milk, plate cultures were made in litmus sugar gelatin.

In plating any sample of milk it is of course a problem to know just what dilution to use in order to obtain the proper number of colonies in the culture plate. In order to be sure of getting satisfactory results two or three different dilutions were used in plating the milk, duplicate plates being made from each dilution. In most experiments all the plates developed with satisfactory numbers and the average of all was taken as representing the number of bacteria present in the milk, so that the figures given in this report represent the averages from the four or six plates made by the two or three dilutions from the same sample of milk. As soon as the plate cultures were made they were placed in an incubator in which a constant temperature of 70° F. was maintained. Here the cultures were allowed to develop for five days, at the end of which time they were studied for the following points:

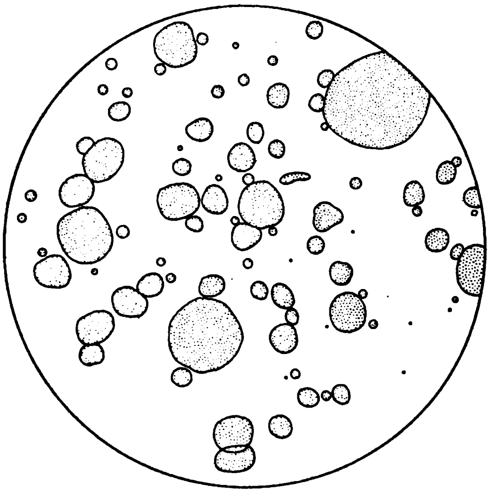

FIG. 1.—Bacteria in atmosphere of barn at farm No. 1. Drawing from photograph of gelatin plate exposed for five seconds in stable at milking time, showing the atmosphere to be well filled with bacteria and molds.

1. The total number of bacteria.
2. The number of acid-producing bacteria.
3. The number of liquefying or enzyme-producing bacteria.

The total number of bacteria indicates the amount of the contamination the milk has received. The number of acid-producing organisms was determined separately from the others because of their importance in connection with the keeping properties of the milk. The group of liquefying organisms includes those species which gain access to the milk thru filth and tend to produce putrefactive changes in it. It is this group especially which it is desirable to exclude from milk intended for human consumption, since it is believed that members of this group are associated with certain digestive troubles, especially in children. It was believed that a study of this group might show interesting comparisons between hand-drawn and machine-drawn milk. The comparative data upon the keeping quality of the different samples of milk were obtained by taking subsamples as soon as the milk reached the laboratory, one of these subsamples being kept at a constant temperature of 70° F. and the other placed in an ice chest where the temperature did not vary greatly from 50° F., or that which is commonly maintained in home refrigerators.

FIRST TREATMENT OF THE MILKING MACHINES.

As previously stated, one of the points which the writer wished to determine was the amount of care necessary for the proper cleansing of the machines after each milking. For this purpose the treatment which the machines received was varied from time to time during the period covered by the tests. The treatment which the machines received is used as a basis for grouping and tabulating the results, and the effect of the various methods used in washing the machines will be brought out in the succeeding tables.

In the first place, it was desired to determine the efficiency of the method already in operation on the farm for washing the machines. This method was as follows: After the morning milking the machines were washt in water to which had been added some sal soda or other washing powder. They were then rinsed by pumping hot water thru them. After the night milking they were simply rinsed with cold water. The machines were not taken apart. The germ content of the milk drawn with the machines treated in this way, in comparison with milk drawn by hand, is shown in Table 4.

TABLE 4.—*Comparison of results of hand and machine milking—machines washt after morning milking with water and sal soda, then rinsed with warm water; after night milking, simply rinsed with cold water—machines not taken apart.*

Date.	No. of experiment.	Time of milking.	Method of milking.	Number of bacteria per cubic centimeter of milk.			Hours to curdling at—	
				Total bacteria.	Acid bacteria.	Liquefying bacteria.	70° F.	50° F.
July 22	3	Morning	Machine	105,000	....	....	35	[a]65
	4	do	Hand	33,233	4,233	3,466	35	[a]65
July 23	7	do	Machine	(b)	....	....	32	[a]41
	8	do	Hand	169,200	145,400	16,425	28	[a]41
July 24	11	do	Machine	1,046,400	1,043,650	2,750	39	[a]65
	12	do	Hand	79,725	16,400	8,225	39	[a]65
July 25	15	do	Machine	812,100	811,400	700	49	104
	16	do	Hand	353,150	350,750	1,150	49	104
July 26	23	Night	Machine	1,571,200	....	....	28	67
	24	do	Hand	988,350	988,350	6,025	45	76
July 27	25	Morning	Machine	1,543,200	1,536,025	7,175	51	49
	26	do	Hand	215,900	188,600	21,775	[a]41	57
July 27	27	Night	Machine	9,417,600	9,417,400	200	60	74
	28	do	Hand	4,179,200	4,107,900	52,600	[a]54	53
July 28	31	Morning	Machine	1,131,200	1,122,275	8,925	37	50
	32	do	Hand	128,300	15,600	9,950	37	49
Average			Machine	2,790,100	2,786,150	3,950	41	64
			Hand	768,382	727,154	14,952	41	64

[a] Curdled at night; exact time not determined.
[b] Too numerous to count with the dilution used.

It will be noticed, in the column marked "Total bacteria," that the machine-drawn milk contained in every case decidedly larger numbers of bacteria than did the milk drawn by hand at the same milking. The numbers obtained in the hand-drawn milk are about what might be expected from the ordinary method of hand milking in a barn of this grade, but the numbers in the machine-drawn milk are very much higher than they should be in fresh milk. It will be noticed also, by the study of the column marked "Acid bacteria," that the bacteria in the machine-drawn milk were chiefly acid-producing organisms, in many cases as high as 99 per cent being such. This proportion of acid bacteria is much larger than would result from ordinary stable contamination, and indicates that the machines were not being properly cleaned and that they furnished a breeding place for the acid-producing species. This would result in the milk that was drawn thru these machines becoming heavily inoculated with acid organisms. It is most significant to note that in spite of the extremely high total number of bacteria in the machine-drawn milk the number of liquefying organisms is in nearly every case very much smaller than in the corresponding hand-drawn milk. When we remember that it is this group which contains putrefactive organisms which get into milk principally from stable filth, and that these organisms producing putrefactive fermentations in the milk are believed to cause serious digestive troubles with children, the significance of these figures is at once apparent. As a result of the machine-drawn milk being protected from external contamination the number of organisms of this group which gained access to the milk was very materially reduced. At the close of this period, after it was thoroly demonstrated that the care which the machines were receiving was not sufficient to keep them in a sterile condition and that they served as breeding places for bacteria, a different method of cleansing was adopted.

SECOND TREATMENT OF THE MACHINES.

All the rubber tubes and teat cups were taken apart. It was found that the long tubes contained considerable quantities of decaying milk, thus making them decidedly insanitary. In order to cleanse these they were run thru a clothes wringer twice in order to loosen and squeeze out any dirt that might be in them. All parts of the machine were then placed in a boiler and thoroly boiled. After the boiling the parts were placed in a weak brine solution, where they remained until milking time. Another series of tests was then made, during which time the machines received the following treatment: After each milking the machines had cold water pumped thru them immediately. They were then washed with hot water containing washing powder, rinsed in boiling water, and placed in brine until needed for the next milking. The results obtained from the tests made while the machines received this treatment are given in Table 5, the first part showing the results from the morning milkings and the second part the results from the night milkings.

TABLE 5.—*Comparison of results of hand and machine milking—machines taken apart and cleaned; rinsed in cold water, then hot sal soda water, then in boiling water, and placed in brine till used; rinsed just before use.*

NIGHT MILKING.

Date.	No. of experiment.	Method of milking.	Number of bacteria per cubic centimeter of milk.			Hours to curdling at—	
			Total bacteria.	Acid bacteria.	Liquefying bacteria.	70° F.	50° F.
July 28	33	Machine	347,800	324,050	13,300	43	50
	34	Hand	420,750	382,250	31,500	32	39
July 29	41	Machine	39,500	10,000	9,000	a 30	a 30
	42	Hand	......	......	......	a 30	49
July 30	45	Machine	622,000	138,000	10,500	36	63
	46	Hand	1,036,000	128,000	16,500	36	a 54
August 2	65	Machine	30,500	18,333	1,041	42	a 102
	66	Hand	681,500	127,000	82,750	a 30	88
August 3	71	Machine	332,000	69,000	57,332	a 30	100
	72	Hand	633,500	500,500	31,375	a 30	93
August 4	77	Machine	223,750	135,750	10,625	a 30	76
	78	Hand	1,302,666	964,500	279,000	28	65
August 5	83	Machine	242,250	97,000	52,625	a 30	69
	84	Hand	582,500	355,500	99,500	a 30	68
August 6	89	Machine	781,500	160,750	74,125	41	91
	90	Hand	197,500	51,000	13,375	a 30	67
Average	......	Machine	327,412	119,110	27,818	35	73
		Hand	693,488	372,687	79,143	31	65
Percentage	......	Machine	......	36.4	8.5	......	......
		Hand	......	53.7	11.4	......	......

MORNING MILKING.

Date.	No. of experiment.	Method of milking.	Total bacteria.	Acid bacteria.	Liquefying bacteria.	70° F.	50° F.
July 29	37	Machine	89,400	57,100	2,450	30	a 41
	38	Hand	113,700	67,300	2,800	27	37
August 3	67	Machine	19,000	6,625	375	a 41	113
	68	Hand	117,125	66,375	9,250	34	85
August 4	73	Machine	52,875	28,000	3,875	32	65
	74	Hand	84,500	39,250	7,125	38	76
August 5	79	Machine	215,750	82,750	36,125	37	......
	80	Hand	300,500	191,250	36,375	29	......
August 6	85	Machine	249,250	56,000	34,625	36	a 65
	86	Hand	558,000	341,500	106,250	36	a 65
August 7	91	Machine	295,500	256,000	13,750	32	78
	92	Hand	266,500	227,500	19,125	29	51
Average	......	Machine	153,629	81,079	15,200	35	72
		Hand	240,054	155,529	30,152	32	63
Percentage	......	Machine	......	52.8	9.9	......	......
		Hand	......	64.8	12.6	......	......

a Curdled at night—exact time not determined.

A comparison of the column marked "Total bacteria" in the above table with the corresponding column in Table 4 shows a very marked diminution in the bacteria found in the machine-drawn milk. While the average for the machine-drawn milk in Table 4 was in round numbers 2,790,000, the average in Table 5 was 327,400 for the night milking and 153,600 for the morning milking. At the same time that this decided falling off in the numbers of bacteria in the machine-drawn milk occurred, the bacteria in the hand-drawn milk in the night samples, as shown in Table 5, was not materially smaller than in Table 4, while in the morning milk the numbers were decidedly smaller. These differences are no greater than would be expected in any series of bacterial tests taken under similar circumstances. In the morning's milk, as shown in Table 5, the numbers of bacteria were also decidedly smaller in the machine-drawn milk. The most important fact to be noted here is the changed relation existing between the bacteria in the machine-drawn and hand-drawn milk. As shown in Table 4, before the machines were cleaned the milk drawn thru them contained many times more bacteria than did the hand-drawn milk, while after the cleaning, and with the brine treatment, the machine-drawn milk contained approximately only one-half as many bacteria as were contained in the milk drawn by hand.

A study of the acid-forming organisms reveals the fact that it was in this group that the decrease in numbers took place. While in the machine-drawn milk, in Table 4, there was an average of over 99 per cent of acid-producing organisms, in Table 5 the percentage of these bacteria was 36.4 (night) and 52.8 (morning). This decrease in the acid-forming bacteria in the machine-drawn milk in this series of tests completely reversed the relation existing between the bacteria found in the machine-drawn and hand-drawn milk, as shown in Table 4. In this series of tests it will be noticed that in almost every case the machine-drawn milk contained decidedly smaller total numbers of bacteria than did the corresponding hand-drawn milk. The same relation exists in the acid organisms, the machine-drawn milk containing both a lower total number and a lower percentage of acid organisms than did the corresponding hand-drawn milk. The night's milk as shown in Table 5 contained an average of 36.4 per cent of acid organisms for the machine-drawn milk as against 53.7 per cent for the hand-drawn milk, and in the morning's milk these percentages were 52.8 for the machine-drawn and 64.8 for the hand-drawn milk. Essentially the same relation in regard to the liquefying organisms in the machine-drawn and hand-drawn milk is shown in Table 5 as was shown in Table 4. The average percentage

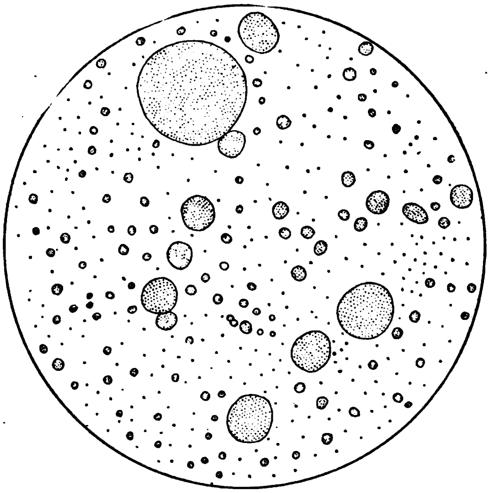

FIG. 2.—Bacteria in machine-drawn milk (426 colonies per plate) in experiment No. 37 (see Table 5).

of liquefiers in the machine-drawn milk of the night samples was 8.5, while it was 11.4 in the hand-drawn milk. In the morning's milk, that drawn by machine contained an average percentage of 9.9 and the hand-drawn 12.6 of liquefiers. The principal value of these two tables lies in the fact that they show such a marked decrease in the bacteria found in the machine-drawn milk, this decrease being the result of the greater care used in the washing of the machine.

THIRD TREATMENT OF THE MACHINES.

In spite of the greatly reduced number of bacteria obtained in the machine-drawn milk, the writer believed that the machines were not being properly sterilized. To test this, cultures were made by shaking sterile water in the rubber tubes after they had been washed as usual. The plate cultures made in this way showed that the short tubes and the ends of the long ones contained only small numbers of bacteria, but that the central part of the three long rubber tubes was still filled with bacteria, which were not being killed or removed by the method used for cleaning the machines.

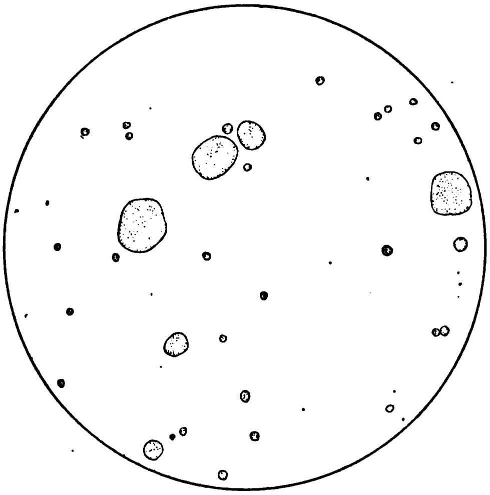

FIG. 3.—Bacteria in machine-drawn milk (43 colonies per plate) in experiment No. 101 (see Table 6).

A further test of this point was made in connection with experiments 77 and 78 by milking two cows with an entirely new machine. The milk thus obtained showed a germ content of 17,612 bacteria per cubic centimeter. At the same time the milk drawn into the machine which was in regular use contained 223,750 bacteria. This great difference in numbers of bacteria found in the milk drawn into the old and the new machines, together with the fact that such large numbers were found in the tubes used on the old machine, was considered definite proof that the daily care used in cleaning the machines was not sufficient to prevent their becoming breeding places for bacteria. Another change was therefore made in the treatment of the machines, and the results obtained are given in Table 6.

On these days the machines were rinsed with cold water as usual. They were then placed in water containing a small amount of powdered borax and boiled for at least one-half hour. The machines were then taken out and hung up in the open air until needed for use.

This treatment was given the machines on but two days. As shown in the table, the average for the machine-drawn milk was 28,562 and for the hand-drawn 48,125 bacteria per cubic centimeter. This is a much smaller number than had been previously obtained by either the machine or hand method, but the relative numbers are not materially different from those found in the previous series of experiments where the machines were soaked in brine. These results seem to indicate that the boiling in a weak borax solution and the soaking in the brine were about equally effective in freeing the machines of bacteria.

TABLE 6.—*Comparison of results of hand and machine milking—machines rinsed with cold water, boiled in water containing borax, then hung up in open air till used.*

Date.	No. of experiment.	Time of milking.	Method of milking.	Number of bacteria per cubic centimeter of milk.			Hours to curdling at—	
				Total bacteria.	Acid bacteria.	Liquefying bacteria.	70° F.	50° F.
August 7	95	Night	Machine	35,125	4,625	11,125	[a]30	86
	96	do	Hand	45,000	9,125	16,625	41	96
August 8	101	do	Machine	22,000	3,000	4,500	[a]54	111
	101A	do	do	7,875	0	0		
	102	do	Hand	51,250	13,500	17,000	43	75
Averages			Machine	28,562	3,812	7,812	42	99
			Hand	48,125	11,312	16,812	42	86
Percentages			Machine		13.4	27.4		
			Hand		23.5	35.0		

[a] Curdled at night—exact time not determined.

FOURTH TREATMENT OF THE MACHINES.

On the night when samples 101 and 102 were taken, a third sample—marked in the table "101A"—was obtained by milking two cows into a machine, all parts of which had been placed in a box and subjected to live steam for 20 minutes. The results obtained in this way are very striking. The number of bacteria contained in the milk thus taken was 7,875, while the number from the other machines was 22,000 and in the milk drawn by hand 51,250.

FIFTH TREATMENT OF THE MACHINES.

At this point another change in the treatment of the machines was made. After being used at night they were rinsed with cold water, then washed with hot water, without the addition of any borax. The results obtained when the machines were thus treated are given in Table 7. After the morning milking the machines were rinsed with cold water as usual. They were then washt in hot water containing borax, after which all the rubber parts were placed in brine until needed for the evening milking. The results obtained from this treatment are shown in Table 8.

TABLE 7.—*Comparison of results of hand and machine milking—machines washt in cold water and then in hot sal soda water, no borax being used.*

Date.	No. of experiment.	Time of milking.	Method of milking.	Number of bacteria per cubic centimeter of milk.			Hours to curdling at—	
				Total bacteria.	Acid bacteria.	Liquefying bacteria.	70° F.	50° F.
August 8	97	Morning	Machine	268,666	49,666	77,999	37	83
	98	do	Hand	296,250	122,000	103,375	28	63
August 9	103	do	Machine	5,875	500	625	38	96
	104	do	Hand	105,500	28,000	32,250	32	63
August 10	109	do	Machine	14,000	2,000	4,125	35	58
	110	do	Hand	102,250	5,875	19,375	30	[a] 41
August 11	115	do	Machine	72,375	5,125	6,625	37	[a] 65
	116	do	Hand	54,625	15,750	9,625	37	[a] 65
August 12	121	do	Machine	64,125	7,125	9,250	[a] 41	(b)
	122	do	Hand	944,000	326,000	92,000	26	[a] 41
August 13	127	do	Machine	84,375	4,000	6,375	35	(b)
	128	do	Hand	25,375	4,625	1,525	35	(b)
August 14	133	do	Machine	76,750	1,625	3,000	[a] 41	(b)
	134	do	Hand	42,625	2,375	250	35	(b)
Average			Machine	83,738	10,006	15,428	38	76
			Hand	224,375	78,089	36,914	32	58
Percentage			Machine		12.0	18.2		
			Hand		32.2	16.5		

[a] Curdled at night—exact time not determined.
[b] Not curdled by August 19.

In four of the experiments shown in this table the machine-drawn milk contained a much smaller number of bacteria than did the corresponding hand-drawn milk. In some of these cases the difference is very great, especially in experiments 103 and 104, and 109 and 110, where the numbers in the machine-drawn milk were small. In the other three experiments, however, the machine-drawn milk contained larger numbers of bacteria than did that drawn by hand. Still, the averages are decidedly in favor of the machine-drawn milk.

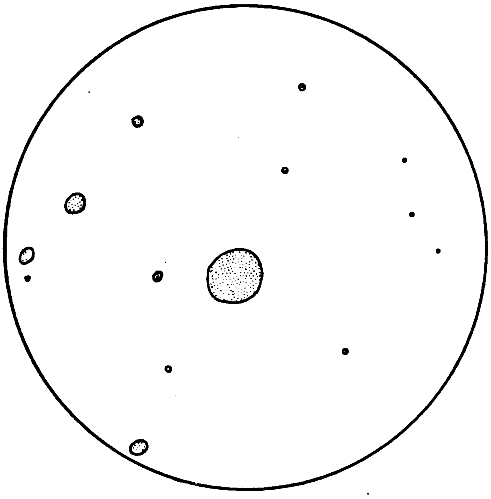

FIG. 4.—Bacteria in milk drawn by steam-sterilized machine (14 colonies per plate) in experiment No. 101A (see Table 6).

The results obtained from the night's milk where the machines were washt in borax water and soaked in brine will be found in Table 8.

TABLE 8.—*Comparison of results of hand and machine milking—machines washt in cold water, then in borax water, and all rubber parts placed in brine till used.*

Date.	No. of experiment.	Time of milking.	Method of milking.	Number of bacteria per cubic centimeter of milk.			Hours to curdling at—	
				Total bacteria.	Acid bacteria.	Liquefying bacteria.	70° F.	50° F.
August 9	107	Night	Machine	15,750	2,250	3,500	43	87
	107A	do	do	2,375	1,250	1,250	68	([b])
	108	do	Hand	115,500	40,500	34,625	38	71
August 10	113	do	Machine	31,750	13,000	7,375	50	101
	113A	do	do	8,750	750	1,375	[a]78	([b])
	114	do	Hand	56,625	7,125	22,250	38	73
August 11	119	do	Machine	49,625	6,500	15,875	[a]30	76
	119A	do	do	19,250	3,625	1,750	[a]54	([b])
	120	do	Hand	115,250	42,250	25,375	37	[a]54
August 12	125	do	Machine	17,750	1,625	2,250	45	([b])
	125A	do	do	13,500	500	2,666	45	([b])
	126	do	Hand	9,125	2,250	625	49	([b])
August 13	131	do	Machine	1,213,000	12,250	11,875	89	([b])
	132	do	Hand	49,875	7,000	0	([b])	([b])
August 14	137	do	Machine	27,625	1,875	1,000	([b])	([b])
	137A	do	do	21,000	250	1,625	([b])	([b])
	138	do	Hand	4,625	625	0	50	([b])
Average			Machine	225,917	6,250	8,125	42	88
			Machine A	12,975	1,275	1,733	61	([b])
			Hand	58,500	16,625	13,812	41	66
Percentage			Machine		2.7	3.6		
			Machine A		9.9	13.4		
			Hand		28.4	23.6		

[a] Curdled in night—exact time not determined.

[b] Not curdled by August 19.

In a part of these experiments the results are very much in favor of the machines, while in the last three tests the comparison is in favor of the hand-drawn milk. This seems to be due to an unusually small number of bacteria in the hand-drawn milk rather than to any marked increase in the number in the machine-drawn milk, with the one exception of experiment 131. In this case it is evident that something decidedly wrong must have occurred to give the milk such an abnormally high germ content. If we leave this one experiment out of the averages we have for the machine-drawn milk an average of 28,500, instead of 225,900, and for the hand-drawn milk 60,225, instead of 58,500. This would give a showing greatly in favor of the machines, as is the case in the preceding tables.

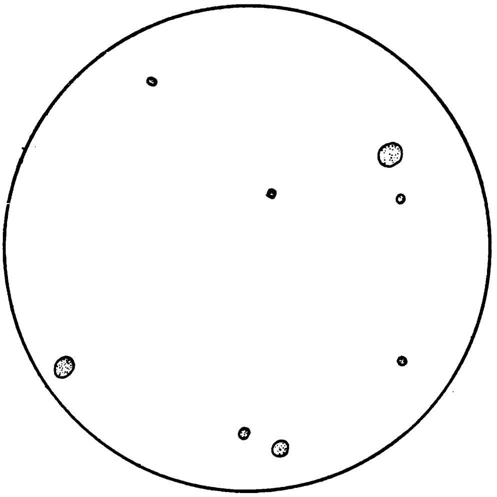

FIG. 5.—Bacteria in machine-drawn milk (8 colonies per plate) in experiment No. 103 (see Table 7).

SIXTH TREATMENT OF THE MACHINES.

In connection with the experiments shown in the preceding table, 2 cows were milked each night with a machine which had been subjected to live steam for 20 to 30 minutes. The results of the milk thus obtained are seen in the experiments marked "A" in the table. It will be seen that the numbers of bacteria in this milk are very much smaller than those obtained by the other machines, running as low as 2,375 bacteria per cubic centimeter in experiment 107A. These results seem to indicate that, even with the care that the regular machines were receiving, they still harbored relatively large numbers of bacteria which contaminated the milk drawn thru them. It will also be noticed that, even with the machine receiving the steam treatment, the germ content of the milk increased quite rapidly from day to day until it was about the same as in the regular machine-drawn milk. This is regarded as an indication that the steam treatment given was not sufficient to completely sterilize this machine, so that the bacteria gradually accumulated in the tubes. It was not possible to get any pressure in the box used for steaming this machine, and it is very probable that the temperature of the inside of the long rubber tubes did not reach that of the steam, and hence did not become sufficiently heated to kill the bacteria in them.

KEEPING QUALITIES OF THE MILK.

As stated at the beginning of this discussion, an attempt was made to determine the keeping qualities of the milk by taking two subsamples as soon as the milk reached the laboratory and keeping one of these at a constant temperature of 70° F. and the other at 50° F. During the day these samples were watched and the time at which they curdled was recorded. It was, of course, impossible to watch the samples during the night, so that in the case of many of them the exact time of curdling could not be determined. Where the samples curdled during the night the time of curdling was arbitrarily given as midnight, and all such results are indicated in the table.

The temperature of 70° F. was taken as representing fairly well the ordinary room temperature during the warm season, and that of 50° F. as representing the normal temperature of the refrigerator in the household.

The records of the time of curdling for the different samples are given in the various tables. It is not always easy to determine why a certain sample of milk should curdle when it does. In general, normal milk becomes sour and curdles in proportion to the number of bacteria contained, but this relation is true only between wide limits. Not infrequently one sample containing twice the number of bacteria contained by another will remain uncurdled the longer time. There are, therefore, marked irregularities when we attempt to harmonize the keeping quality with the germ content. However, taking a series of samples containing a high germ content and comparing with another series with a comparatively low germ content, it will be found that there is an inverse relation existing between the germ content and the keeping quality. Frequently the species of organisms which the milk contains has more to do with determining its keeping quality than does the actual number of bacteria which may be in the milk. This fact accounts for many of the apparent discrepancies between the germ content and the keeping quality. While the results given in the preceding tables show many irregularities, yet they are of considerable interest, and in the long run are of value as an indication of the keeping quality of the different samples of milk.

SUMMARY OF EXPERIMENTS AT FARM NO. 1.

In Table 9 are given the averages for the germ content and keeping quality from the six preceding tables. While these averages are not of as much value as the results of the individual experiments, they give in a general way the relation existing between the machine-drawn and hand-drawn milk.

It will be noticed in this table that there is a difference in favor of the keeping quality of the machine-drawn milk, both in the samples kept at 70° F. and in those kept at 50° F., the greater difference in favor of the machine milk being shown in the samples which were kept at 50° F. One important fact which is shown in this table is the greatly increased keeping quality of the milk which was drawn into the steam-sterilized machine marked "A" in the table. These samples held at 70° F. kept on the average one-third longer than the average of the ordinary machine-drawn or hand-drawn milk, while those held at 50° F. had not curdled by August 19 when the test ended. This emphasizes the importance and indicates the possibilities of greatly increasing the keeping quality by thoroly sterilizing the machine.

TABLE 9.—*Summary of germ content and keeping quality of milk at farm No. 1.*

No. of table.	Number of experiments.	Method of milking.	Total number of bacteria found.	Hours to curdling at—	
				70° F.	50° F.
4	8	Machine	2,790,100	41	64
	8	Hand	768,382	41	61
5	8	Machine	327,412	35	73
	8	Hand	693,488	31	65
	6	Machine	153,629	35	72
	6	Hand	240,054	32	63
6	2	Machine	28,562	42	99
	2	Hand	48,125	42	86
7	7	Machine	83,738	38	76
	7	Hand	224,375	32	58
8	6	Machine	225,917	42	88
	5	Machine A	12,975	61	(a)
	6	Hand	58,500	41	66

[a] Not curdled by August 19.

EXPERIMENTS AT FARM NO. 2.

The conditions of cleanliness existing in this stable are considerably above the average (fig. 6.) From a sanitary standpoint this stable was not as well constructed as was the one at farm No. 1. The stable is entirely below ground on the west and north sides, but receives a good supply of light from the south and east. The feeding alley and mangers are made of cement, but the stable floor, the drop, and the main floor behind the cows are made of plank. While the construction of the stable itself is not especially suited to the production of clean milk, considerable care was taken to keep the stable and the cows as clean as possible. Considerably more than average care was exercised in the milking and the subsequent handling of the milk, so that this stable may properly represent a fairly sanitary dairy. It is not to be expected that the germ content of milk produced in such a stable could be kept as low as in a stable constructed on more approved sanitary plans, but the number of bacteria found in the hand-drawn milk, as given in the following tables, shows, however, that the sanitary conditions of the stable at milking time were excellent. The stable floor was always swept twenty minutes to one-half hour before milking was commenced, and just before milking, the udders were wiped with a damp cloth.

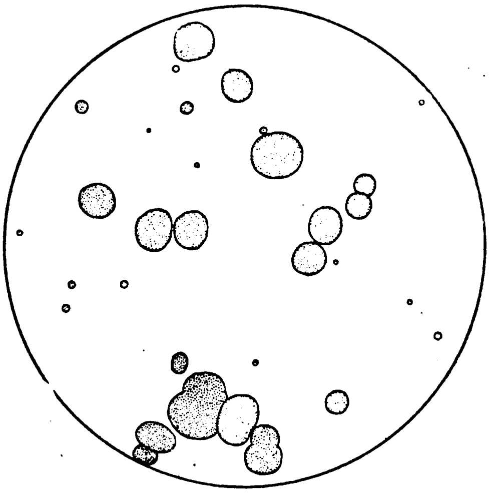

FIG. 6.—Bacteria in atmosphere of barn at farm No. 2. Drawing from photograph of gelatin plate exposed for twenty seconds in stable at milking time, showing the low germ content of the atmosphere, 34 colonies per plate.

METHOD OF EXPERIMENT.

In beginning the experiments in this dairy it was desirable, first, to ascertain the relative germ content existing in the machine-drawn and hand-drawn milk. For this purpose the herd was divided into two lots of 4 cows each. One of these groups was milked by the machine and the other by hand into an open pail in the ordinary way. As soon as the milking was completed, samples were taken from the mixt milk of each group of cows and these were carried to the laboratory for testing. The details of these experiments are the same as those already outlined in connection with the experiments at farm

No. 1. In this series of tests samples were taken at the night milking only and were placed on ice until the next morning, when the plate cultures were made. It is probable that the figures given in the tables are slightly lower than would have been obtained had the samples been plated immediately, since there is usually a dropping out of certain species during the first few hours after drawing the milk. In a few cases where the samples were plated at night and again the next morning this was shown to be true.

FIRST TREATMENT OF THE MACHINES.

At the outset it was desired to ascertain the efficiency of the milking machine with the treatment it was regularly receiving on the farm. Three days' tests were made without altering the treatment which the machines received and the results were compared with those obtained from the corresponding samples drawn by hand. The results of these tests are given in Table 10.

TABLE 10.—*Comparison of results of hand and machine milking—machines rinsed with cold water, washt with warm water containing sal soda, then rinsed with hot water.*

Date.	Number of experiments.	Method of milking.	Bacteria per cubic centimeter of milk.					Hours to curdling at—	
			Total bacteria.	Acid bacteria.	Per cent of acid bacteria.	Liquefying bacteria.	Per cent of liquefying bacteria.	70° F.	50° F.
July 21	2	Machine	69,130	1,513	2	683	1	62	110
	1	Hand	24,100	....	....	2,325	10	40	100
July 22	5	Machine	8,143	7,213	89	1,400	17	76	165
	6	Hand	1,900	492	26	100	5	76	147
July 23	9	Machine	441,600	439,000	99	1,850	0	52	97
	10	Hand	2,200	570	26	150	7	52	86
Averages	....	Machine	172,958	149,242	....	1,311	....	63	124
	....	Hand	9,400	531	....	858	....	56	111

During this period the machines were treated as follows: After milking they were rinsed by pumping cold water thru them. This was followed by warm water containing some washing powder, commonly sal soda. They were then rinsed with clean hot water and hung up in the air until needed for use at the next milking. A glance at the "Total bacteria" column in the table shows the same relation existing between the bacteria found in the hand-drawn and machine-drawn milk as was found in the experiments at farm No. 1. The number of bacteria in the machine-drawn milk was decidedly higher than in the corresponding hand-drawn milk in each experiment, the average for the three samples of machine-drawn milk being 172,958 bacteria per cubic centimeter, while the average for the hand-drawn milk is 9,400. It will also be seen that the organisms in the machine-drawn milk were largely acid-producing species. This also corresponds to the conditions found at farm No. 1. It is evident that the milk became contaminated with large numbers of acid-producing organisms while passing thru the machines. These results seem to prove beyond question that the care which the machines were regularly receiving was not sufficient to keep the tubes in a sterile condition and they had therefore become breeding places for bacteria, which were washt into the milk when the machines were used.

SECOND TREATMENT OF THE MACHINES.

At this point a slight change was made in the method of treating the machines, and the figures given in Table 11 show the results of two days' tests with the machines thus treated.

TABLE 11.—*Comparison of results of hand and machine milking—treatment of machine the same as indicated for Table 10, except that it was scalded just before use by pumping boiling water thru it.*

Date.	No. of experiment.	Method of milking.	Bacteria per cubic centimeter of milk.					Hours to curdling at—	
			Total bacteria.	Acid bacteria.	Per cent of acid bacteria.	Liquefying bacteria.	Per cent of liquefying bacteria.	70° F.	50° F.
July 24	[a]13	Machine	226,700	8,883	3	2,287	1	76	130
	14	Hand	11,850	2,600	22	250	2	[b]54	[b]102
July 26	21	Machine	12,000	4,988	42	487	4	97	171
	22	Hand	1,100	475	43	112	10	64	171
Average		Machine	119,350	6,935		1,387		87	151
		Hand	6,475	1,537		181		59	137

[a] Udders not washt.

[b] Curdled at night—exact time not determined.

The machines were washt as previously described, but they were given an additional scalding just before being used by pumping boiling water thru them. It was hoped that this might dislodge and wash out any bacteria which might have accumulated in the tubes during the day, thus reducing the germ content of the milk. This additional scalding, however, apparently had little effect upon the condition of the tubes, since the numbers of bacteria were not materially decreased, as will be seen by a study of Table 11. Both the total number and the number of acid-producing organisms and also the liquefying bacteria continued to be much more numerous in the machine-drawn milk than in that drawn by hand.

It will be noted that in experiment 13 the udders were not wiped with a damp cloth previous to milking. This fact, no doubt, at least partially accounts for the high numbers of bacteria found in this lot of milk.

THIRD TREATMENT OF THE MACHINES.

After being washt as previously described, the rubber tubes and teat cups were placed in water and boiled for three-quarters of an hour. They were then hung up in the open air until needed for the next milking. Just before using they were rinsed as in the previous experiments by having boiling water pumped thru them. Table 12 gives the results of four tests taken with the machines thus treated.

TABLE 12.—*Comparison of results of hand and machine milking—machines rinsed with cold water and washt with sal soda, then boiled for three-quarters of an hour and rinsed with boiling water just before use.*

Date.	No. of experiment.	Method of milking.	Bacteria per cubic centimeter of milk.					Hours to curdling at—	
			Total bacteria.	Acid bacteria.	Per cent of acid bacteria.	Liquefying bacteria.	Per cent of liquefying bacteria.	70° F.	50° F.
July 27	29	Machine	18,737	3,025	16	612	3	91	74
	30	Hand	781	125	10	137	18	[a]126	[a]150
July 28	35	Machine	5,612	50	1	100	2	144	[a]54
	36	Hand	6,537	462	7	50	1	[a]54	[a]126
July 29	39	Machine	85,700	82,950	97	1,750	2	[a]78	134
	40	Hand	7,175	75	1	2,200	3	50	87
July 30	47	Machine	....	....	....	....	....	[a]54	[a]102
	48	Hand	10,350	3,050	30	1,000	9	52	171
Average	....	Machine	36,683	28,675	....	1,231	....	91	91
		Hand	6,211	985	....	847	....	71	133

[a] Curdled at night—exact time not determined.

This increased treatment of the machine apparently was not sufficient to greatly change the quality of the machine-drawn milk. However, in experiment 35 the number of bacteria in the machine-drawn milk was somewhat less than in the corresponding hand-drawn sample. It should be borne in mind that the numbers of bacteria obtained by the hand milking are extremely low for milk drawn into an ordinary open pail. These low numbers of bacteria found in the hand-drawn milk emphasize the fact that the atmosphere in the stable was relatively free from organisms, and that considerable care was exercised in the drawing and handling of the milk. The man who did the milking was unusually careful in all his operations. In experiment 39 the rubber tube connecting the pulsator with the stanchion cock was not boiled with the other tubes, and it is probable that this furnished the source of contamination and explains the very high number of bacteria in this lot of milk. In order to determine whether the rubber tubes were responsible for the larger numbers of bacteria in the machine-drawn milk a new set of tubes was used in experiments 63 and 69. These new tubes were washt and then scalded, together with the rest of the machine, just before using. This resulted in a smaller number of bacteria from the machine-drawn milk than had been previously obtained.

TABLE 13.—*Comparison of results of hand and machine milking—new tubes and teat cups used, and machines washt as usual and scalded just before using.*

Date.	Number of experiment.	Method of milking.	Bacteria per cubic centimeter of milk.					Hours to curdling at—	
			Total bacteria.	Acid bacteria.	Per cent of acid bacteria.	Liquefying bacteria.	Per cent of liquefying bacteria.	70° F.	50° F.
August 2	63	Machine	3,625	162	4	87	2	86	[a] 126
	64	Hand	650	275	42	25	4	161	206
August 3	69	Machine	[b] 1,637	925	58	25	1	50	116
	70	Hand	1,344	150	12	144	11	[a] 102	[a] 126
Averages		Machine	2,631	543	....	55	....	68	121
		Hand	997	212	....	84	....	132	166

[a] Curdled at night—exact time not determined. [b] Two jets of foremilk removed.

The results of these tests as shown in Table 13 would make a very good showing for the machine were it not for the fact that the numbers in the hand-drawn milk were so extremely low. However, it ought to be possible to obtain milk by means of the machine which would contain a lower germ content than hand-drawn milk under the same conditions provided the parts of the machine thru which the milk past were sterile at the outset, since the milk drawn by means of the machine is entirely shut off from external contamination. It ought to be possible to obtain the milk in the pail with practically nothing but the bacteria which exist in the udder. This manifestly was not the case with the trials made thus far and it was evident that still greater care was necessary in order to completely sterilize the machines.

In order to determine the real condition of the inside of the tubes a test was made at this point to find out whether or not the inside of the rubber tubes was being sterilized by the treatment given them. A small quantity of sterilized water was run into the central part of the long rubber tubes and rinsed back and forth. This was then poured out and a drop of this water inoculated into a gelatin plate. This resulted in the development of 1,728 colonies of bacteria as the germ content of a single drop of the water used for rinsing the tube. This showed beyond a doubt that the inside of the tubes was not being properly cleansed and sterilized by the treatment thus far used and that greater care was necessary in order to overcome this trouble.

FOURTH TREATMENT OF THE MACHINES.

The writer desired especially to test the efficiency of the machine for the production of sanitary milk, or milk containing a minimum germ content. It was therefore decided to take still more vigorous means for cleansing and sterilizing the machines. To do this, in addition to the treatment usually given, the tubes were boiled for three-quarters of an hour in water containing a small quantity of powdered borax. They were then thoroly rinsed with boiling water to remove any traces of borax which might be in the tubes. A small amount of borax was also added to the water used for wiping off the udders. In addition to these precautions a small amount of foremilk was also drawn before attaching the machine to the cows, and the pail was carried out of the stable before the cover was removed. This treatment proved to be more effective than any previously given, as will be seen by a study of Table 14.

TABLE 14.—*Comparison of results of hand and machine milking—machines rinsed with cold water, washt with warm water containing sal soda, boiled three-quarters of an hour in water with borax, and scalded just before use; udders wiped with borax water.*

Date.	No. of experiment.	Method of milking.	Bacteria per cubic centimeter of milk.					Hours to curdling at—	
			Total bacteria.	Acid bacteria.	Per cent of acid bacteria.	Liquefying bacteria.	Per cent of liquefying bacteria.	70° F.	50° F.
August 4	75	Machine..	462	....	....	....	....	62	115
	76	Hand.....	1,550	225	15	419	28	49	139
August 5	81	Machine..	400	12	3	25	6	123	(a)
	82	Hand.....	881	81	9	0	0	[b]54	(a)
August 6	87	Machine..	612	112	18	37	6	[b]78	124
	88	Hand....	1,300	75	6	80	6	48	100
Averages ...	....	Machine..	506	62	....	31	....	88	120
		Hand.....	1,090	78	....	40	....	50	120

[a] Not curdled by August 19.

[b] Curdled at night—exact time not determined.

It will be noticed in this table that the machine-drawn milk contained in each case much smaller numbers of bacteria than did the corresponding hand-drawn milk. These numbers compare very favorably with the germ content of the best grades of "sanitary" or "clinical" milk which are now placed upon the market. In fact these numbers are below the germ content of much of such milk. The results of these tests are sufficient to prove that the germ content of milk can be reduced to a very low figure by the use of the machine when properly sterilized. While the boiling in borax water was apparently an effective treatment, attention should be called to the danger of using this or other antiseptics to rid the rubber tubes and teat cups of bacteria. A very thoro rinsing of the tubes is necessary in order to remove all traces of the antiseptic so that none of it can be taken up by the milk as it passes thru the machine. If the antiseptic were left in the tubes it would be the same in effect as treating the milk with a preservative—a practise that is considered very objectionable. There was also some question in regard to the effect of the boiling upon the life of the rubber tubing, and it was some work to boil the machines in this way each day. For these reasons it was thought desirable to find a simpler way of sterilizing the machines and one which would not have an injurious effect upon the rubber.

FIFTH TREATMENT OF THE MACHINES.

Common salt is a good germicide, and it also has a hardening and preservative effect upon the rubber. It was therefore thought that possibly a brine solution might be used for sterilizing the rubber tubes instead of boiling them in borax water. To test this the machines were washt as usual, after which all the rubber parts were placed in a moderately strong brine solution, where they remained until needed for the next milking. Before being used they were thoroly rinsed by having boiling water pumped thru them. Table 15 gives the results of the tests made with the machines thus treated.

TABLE 15.—*Comparison of results of hand and machine milking—machines being washt as usual, the rubber parts then placed in brine till needed, and scalded just before use.*

Date.	No. of experiment.	Method of milking.	Bacteria per cubic centimeter of milk.					Hours to curdling at—	
			Total bacteria.	Acid bacteria.	Per cent of acid bacteria.	Liquefying bacteria.	Per cent of liquefying bacteria.	70° F.	50° F.
August 7	93	Machine	6,450	475	7	625	10	52	100
	94	Hand	4,075	562	14	363	9	[a] 54	[a] 102
August 8	99	Machine	400	112	28	100	25	48	109
	100	Hand	600	100	16	0	0	68	121
August 9	105	Machine	483	100	21	33	7	101	([b])
	106	Hand	18,750	1,350	7	1,449	8	[a] 78	([b])
Averages	....	Machine	2,444	229	....	253	....	67	105
		Hand	11,712	1,006	....	604	....	67	112

[a] Curdled in night—exact time not determined.

[b] Not curdled August 19.

It will be noticed that in experiment 93 the number of bacteria was decidedly higher than in the preceding tests given in Tables 13 and 14, while in experiments 99 and 105 they were as low as when the machine was treated with borax. Probably the higher number in experiment 93 was due to the fact that only part of the rubber tubes were treated with the brine solution, while on other days all the rubber parts were placed in the brine. It would seem from the results of these tests that the brine treatment is as effective as the boiling in borax water and is at the same time much simpler.

SIXTH TREATMENT OF THE MACHINES.

The usual method for sterilizing dairy utensils is by steam. This is entirely satisfactory with ordinary dairy utensils, but steam is known to have an injurious effect upon rubber, for which reason steam sterilizing was not generally used in this series of experiments; but at this point it was deemed desirable to compare the efficiency of steam sterilizing with the treatments previously given, in order to determine whether or not it was possible to reduce still further the germ content of the machine-drawn milk. To accomplish this a large wooden box was fitted up to serve as a steam sterilizer. After the machine had been washt as usual, it was placed in this box and subjected to live steam for 30 minutes. Table 16 gives the results of this series of tests.

TABLE 16.—*Comparison of results of hand and machine milking—machines washt as usual and sterilized in steam for thirty minutes.*

Date.	Number of experiment.	Method of milking.	Bacteria per cubic centimeter of milk.					Hours to curdling at—	
			Total bacteria.	Acid bacteria.	Per cent of acid bacteria.	Liquefying bacteria.	Per cent of liquefying bacteria.	70° F.	50° F.
August 10	111	Machine	560	37	7	0	....	77	(a)
	112	Hand	400	112	28	75	19	158	(a)
August 11	117	Machine	1,962	337	18	75	4	69	(a)
	118	Hand	1,762	375	22	287	17	[b] 30	60
August 12	123	Machine	1,087	137	14	75	7	45	(a)
	124	Hand	850	100	12	112	13	49	(a)
August 13	129	Machine	9,887	225	2	375	4	(a)	(a)
	130	Hand	5,540	860	10	250	5	[b] 78	(a)
August 14	135	Machine	3,750	387	10	162	4	[b] 54	(a)
	136	Hand	1,337	362	28	275	21	(a)	(a)
Averages	....	Machine	3,449	225	....	137	....	64	....
		Hand	1,978	362	....	200	....	79	....

[a] Not curdled August 19. [b] Curdled at night—exact time not determined.

It will be noticed that in each test the machine-drawn milk contained larger numbers of bacteria than did the corresponding hand-drawn sample. It was impossible, with the equipment used, to get any pressure in the steam chest, hence the temperature did not quite reach the boiling point (212° F.). Under these conditions it is probable that the inside of the rubber tubes did not become heated to the temperature of the steam, and they, therefore, were not sterilized during the thirty minutes that they were subjected to the steam treatment. Probably a longer steaming might prove effective, but it is evident that the treatment here given was not sufficient to completely sterilize the tubes. The rubber tubing did not appear to be seriously injured by the steam treatment during this series of tests, but it is quite probable that long-continued steam sterilizing would injure the rubber. This series of tests would at least indicate that it is necessary to subject the tubes to greater steam pressure or else use other germicides in order to completely sterilize the rubber tubing. At the close of this period one test was made by treating the machine in brine, as already described. The results of this test are shown in Table 17.

TABLE 17.—*Comparison of results of hand and machine milking—machines treated as indicated for Table 15.*

Date.	Number of experiment.	Method of milking.	Bacteria per cubic centimeter of milk.					Hours to curdling at—	
			Total bacteria.	Acid bacteria.	Per cent of acid bacteria.	Liquefying bacteria.	Per cent of liquefying bacteria.	70° F.	50° F.
August 15	141	Machine	13,675	2,463	18	1,056	8	....	....
	142	Hand	2,100	763	36	493	23	62	....

KEEPING QUALITIES OF THE MILK.

The discussion of the keeping qualities of the milk given in connection with the experiments at farm No. 1 will in general apply with equal force to the results obtained at farm No. 2. Practically the same results were obtained in both cases. In the majority of instances the difference in keeping quality is in favor of the machine-drawn milk, both at 70° and 50° F. This difference is especially noticeable in Table 14, where the germ content of the machine-drawn milk averaged the smallest. With an average of 506 bacteria per cubic centimeter the milk kept on an average eighty-eight hours at 70° F., while the hand-drawn milk, with an average of 1,090 bacteria, kept an average of but fifty hours, making an average difference of thirty-eight hours in favor of the machine-drawn milk. This, at least, is an indication of the extent to which the keeping quality of the milk may be improved by the use of the machines, provided they are kept in a sterile condition and care is used in their manipulation.

SUMMARY OF RESULTS OF BACTERIOLOGICAL INVESTIGATIONS.

The results of the foregoing experiments seem to justify the following conclusions:

1. Unless sufficient care is used in cleaning the machines, decaying milk and bacteria accumulate in the rubber tubes and contaminate the milk as it passes thru them.
2. The few dairymen now using these machines are not exercising sufficient care in washing and sterilizing the machines to keep them in sanitary condition; their milk is therefore of poorer quality from the sanitary standpoint than that drawn by hand under the same stable conditions.
3. Good sanitary conditions in a stable may be completely counteracted by the insanitary condition of the milking machine.
4. When kept in fairly clean condition the machine-drawn milk contains decidedly smaller numbers of bacteria than the corresponding hand-drawn milk.
5. When the machines are not well cleaned both the number and percentage of acid-producing bacteria are higher than in the hand-drawn milk, but when they are fairly well cleaned both the total number and the percentage of these bacteria are decidedly lower than in the corresponding hand-drawn milk.
6. Both the total number and the percentage of liquefying bacteria found in the milk were in most cases greatly reduced by the use of the machines. This fact is of special significance in milk designed for direct consumption.
7. When properly cared for, drawing the milk by means of the machine increases its keeping quality.

8. Washing the machines with cold water and then with hot water containing sal soda is not sufficient to keep the rubber tubes clean. Under this treatment the inside of the tubes becomes coated with decaying milk, thus forming ideal conditions for the multiplication of various species of bacteria.

9. Scalding the machines by pumping boiling water thru them just before use had little or no effect in reducing germ content of the milk.

10. Boiling in clear water for three-quarters of an hour was not sufficient to keep the rubber tubes in a sterile condition.

11. Subjection to steam without pressure for thirty minutes was not sufficient to sterilize the rubber tubes.

12. Placing the rubber parts in brine for several hours after being washt reduced the germ content of the machine-drawn milk to about one-half that of the milk drawn by hand.

13. Boiling in water containing a small amount of powdered borax had about the same effect in reducing the bacterial content of the milk as did the brine treatment, but the use of borax is dangerous unless extreme care is exercised.

14. The machines may be very effective in the production of sanitary milk if they are properly cleaned and sterilized.

15. The results of these experiments indicate that the machines may be kept in such an insanitary condition that the keeping quality will not be improved, but may be seriously impaired. They also indicate that with properly cleaned and sterilized machines the keeping quality of the milk may be very materially improved.

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136·h 58

Issued December 31, 1906.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN NO. 93.
A. D. MELVIN, CHIEF OF BUREAU.

THE RELATION OF TUBERCULOUS LESIONS TO THE MODE OF INFECTION.

BY

E. C. SCHROEDER, M. D. V.,
Superintendent of Experiment Station,
Bureau of Animal Industry,

AND

W. E. COTTON,
Expert Assistant at Experiment Station,
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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., November 3, 1906.

Sir: I have the honor to transmit herewith, for publication as a bulletin of this Bureau, an article entitled "The Relation of Tuberculous Lesions to the Mode of Infection," by Dr. E. C. Schroeder and W. E. Cotton, of the Experiment Station of this Bureau.

In the experiments described in this paper, which were carried out mainly for the purpose of further testing the susceptibility of the lung to infection with tubercle bacilli regardless of the point at which the infectious material enters the body, tuberculosis was produced in the lungs of a calf and three hogs by inoculation near the end of the tail. This work, taken in connection with that reported in Bulletins Nos. 86 and 88 of this Bureau, indicates that the lungs may readily become the seat of tuberculous disease no matter thru what channel the bacilli gain entrance into the body, and that the location of lesions in the lungs can no longer be considered as reliable evidence that the infection entered thru the respiration.

Dried sputum has long been regarded as the most prolific agent in the dissemination of tuberculosis among people, but the facts presented in this bulletin, which are believed to have an important bearing on the problem of how tuberculosis is contracted by human beings, go to show that too much importance has been attached to this and not enough to the more serious danger from fresh or moist tuberculous material which enters human food in many ways, one of the commonest of which is attributable to the tuberculous dairy cow.

Respectfully,

A. M. Farrington,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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Issued December 31, 1906.

U. S. DEPARTMENT OF AGRICULTURE,
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A. D. MELVIN, CHIEF OF BUREAU.

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IMPORTANCE OF THE APPARATUS IN CALORIMETRIC DETERMINATIONS.

Not only in physical and chemical laboratories do calorimetric determinations find application, but they have acquired special prominence in connection with physiological investigations and research work, and it is in this line of work that at the present time their application in the agricultural science has been found to be of so great importance. Thus, the very elaborate, time-consuming, and expensive nutrition investigation experiments with cattle by the use of the respiration calorimeter are so planned that the successful solving of the question of energy metabolism in the animal body rests almost entirely upon the bomb calorimeter—upon its efficiency in quickly and accurately determining the heats of combustion of feed and excreta. Being, then, a most important part of the whole plan, and since so much depends upon the work of this apparatus, one is not justified, because much work has been done with it in the past, in assuming that it is perfect in all respects, or that it is adapted alike to all kinds of organic substances. Instead, it should be severely tested from different points of view, and that was the object of this work.

EARLIER DETERMINATIONS OF HEATS OF COMBUSTION.

To begin with, it may be well to look a little into the work already done, and note the condition in which our knowledge of the heat values of organic substances is at the present time, and how well different investigators agree in their determinations. For this purpose, as well as to get a general idea of heat values, I have, without going back to the original source of the information, collected and tabulated a large number of results upon the heats of combustion of the more common organic substances as obtained by various investigators. The values are taken from text-books and scientific reference books, some of which are still in use in schools and elsewhere.

To make the tables as condensed as possible the works or books referred to are represented in one column, each book by a single number, and the investigators in another column by the first letters of their respective names.

TABLE I.—*Heats of combustion of 1-gram substance, expressed in small calories.*

Substance.	Formula.	Calories.	References.[a]	Investigators.[a]
ELEMENTS, ETC.				
Hydrogen	H_2	34,462.0	1,3	F. & S.
Hydrogen (8 investigators)	H_2	[b]34,154.3	10	F. & S.
Do	H_2	[c]34,800.0	10	H.
Carbon	C	7,770.1	10	F. & S.
Charcoal (wood)	C	8,080.0	1,3,10	F. & S.
Charcoal (sugar)	C	7,714.0	10	Gr.
Do	C	8,039.8	10	F. & S.
Do	C	7,965.0	10	Sch.
Diamond (to CO_2)	C	7,859.0	10	B. & P.
Diamond (to CO)	C	2,141.7	10	B.
Iron (to Fe_2O_3)	Fe	1,600.0	9	
Iron (to FeO)	Fe	1,352.6	10	F. & S.
Sulphur	S_2	2,260.3	10	F. & S.
Do	S_2	2,165.6	10	B.
Sulphur (soft)	S_2	2,220.5	10	F. & S.
Do	S_2	2,162.5	3	F. & S.
CO to CO_2		2,436.0	3	F. & S.
Do		2,431.0	10	An.
Do		2,402.7	10	F. & S.
Do		2,438.6	10	B.
Do		2,441.7	10	Th.
Nitrogen to HNO_3 (sol.)	N_2	1,022.6	8	
NO to NO_2		652.3	10	Th.
PROTEIDS, ETC. (ALBUMINOIDS.)				
Gluten		5,990.3	2	B.
Do		6,141.0	2	D.
Elastin		5,961.3	1,2,10	St. & L.
Plant fibrin		5,832.3	2,10	B. & A.
Do		5,941.6	2,10	St. & L.
Do		6,231.0	1,2	D.
Serum albumin		5,917.8	1,2,8,10	St. & L.
Syntonin		5,907.8	2,1	St. & L.
Hemoglobin		5,910.0	2,10	B. & A.
Do		5,914.8	1,3	B. & A.
Do		5,885.1	1,2,3,10	St. & L.
Do		5,950.0	2	Ru.
Do		5,949.0	1,2,3,10	D.
Milk casein		5,626.4	2,10	B. & A.
Do		5,867.0	2,3,8,10	St. & L.
Do		5,629.2	1,3	B. & A.
Do		5,717.0	1,2,3,10	St.
Do		5,785.0	2	D.
Do		5,849.6	2,3,10	St. & L.
Do		5,858.0	1	St. & L.
Do		5,855.0	1	D.
Yolk of egg		8,112.4	2,3	B. & A.
Yolk of egg (fat free)		5,840.9	3,10	St. & L.
Legumin		5,793.1	1,2,10	St. & L.
Do		5,573.0	2	D.
Vitellin		5,780.6	2,10	B. & A.
Do		5,745.1	1,2,3,10	St. & L.
Do		5,784.1	3	St. & L.

[a] Abbreviations:

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St. Stohmann.
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Th. Thomsen.
V. Vieille.

[b] Lowest.

[c] Highest.

TABLE I.—*Heats of combustion of 1-gram substance, expressed in small calories*—Cont'd.

Substance.	Formula.	Calories.	References.	Investigators.
PROTEIDS, ETC. (ALBUMINOIDS)—con.				
Egg albumin		5,687.4	1,2,10	B. & A.
Do		5,735.2	1,2,3,8,10	St. & L.
Do		5,579.0	1,2,3,10	St.
Do		5,690.6	3	St.
Muscle (fat free and water)		5,728.4	2,10	B. & A.
Muscle (extracted)		5,720.5	2,8,10	St. & L.
Muscle (fat free)		5,662.6	2,10	St. & L.
Do		5,324.0	2,10	St.
Muscle (fat free, ash free)		5,656.0	2,10	Ru.
Do		5,640.9	2,10	St. & L.
Do		5,345.0	10	Ru.
Albumin cryst		5,672.0	1,2,10	St. & L.
Do		5,598.0	1,2,10	St.
Blood fibrin		5,529.1	2,10	B. & A.
Do		5,637.1	2,3,10	St. & L.
Do		5,511.0	1,2,3,10	St.
Do		5,709.0	2	D.
Do		5,772.0	1	D.
Do		5,532.4	3	St.
Do		5,567.3	3	St.
Albumin (Harnack's)		5,553.0	2	St. & L.
Wool		5,564.2	2	B. & A.
Do		5,510.2	2	St. & L.
Congluten		5,479.0	2,10	St. & L.
Do		5,362.0	2,10	St.
Fibrin of skin		5,355.1	2,10	St. & L.
Peptone		5,298.8	1,2,3,8,10	St. & L.
Do		5,069.0	2	D.
Do		4,876.0	1	D.
Do		4,914.0	1	D.
Fish glue		5,240.1	2,10	B. & A.
Do		5,493.0	1,2	D.
Chondrin		5,342.4	1,2,3,10	B. & A.
Do		5,130.6	1,2,3,10	St. & L.
Do		4,909.0	2	D.
Ossein		5,410.4	1,2,10	B. & A.
Do		5,039.9	1,2,10	St. & L.
Fibroin		5,095.7	2,10	St. & L.
Do		4,979.6	2,10	St. & L.
Chitin		4,655.0	2,10	St. & L.
Do		4,650.3	2,10	B. & A.
Tunicin		4,146.8	2,10	B. & A.
Paraglobulin		5,637.0	1,2,10	St.
Legumin		5,793.0	1,10	St. & L.
Proteids (mean)		5,730.8	8	
AMIDES, ETC.				
Urea	CON_2H_4	2,530.1	2,10	B. & P.
Do	CON_2H_4	2,541.9	1,2,10	St. & L.
Do	CON_2H_4	2,465.0	1,2,5,10	St.
Do	CON_2H_4	2,537.0	1,2	D.
Do	CON_2H_4	2,523.0	2,10	R.
Do	CON_2H_4	2,121.0	1	F.
Glycocoll	$C_2H_5NO_2$	3,133.6	2,9,10	B. & A.
Do	$C_2H_5NO_2$	3,129.1	2,9,10	St. & L.
Do	$C_2H_5NO_2$	3,050.0	1	St.
Do	$C_2H_5NO_2$	3,053.0	2,10	St.
Alanin	$C_3H_7NO_2$	4,370.7	2,10	B. & A.
Do	$C_3H_7NO_2$	4,355.5	2,10	St. & L.
Leucin	$C_6H_{13}NO_2$	6,536.5	1,2,10	B. & A.
Do	$C_6H_{13}NO_2$	6,525.1	1,2,10	St. & L.
Sarkosin	$C_3H_7NO_2$	4,505.9	1,2,10	St. & L.
Hippuric acid	$C_9H_9NO_3$	5,659.3	1,2,9,10	B. & A.
Do	$C_9H_9NO_3$	5,668.2	1,2,9,10	St. & L.
Do	$C_9H_9NO_3$	5,642.0	2,5,10	St.
Aspartic acid	$C_4H_7NO_4$	2,911.1	2,10	B. & A.
Do	$C_4H_7NO_4$	3,423.0	1	St.
Do	$C_4H_7NO_4$	2,899.0	2,10	St. & L.
Tyrosin	$C_9H_{11}NO_3$	5,915.9	1,2,10	B. & A.
Asparagin	$C_4H_8N_2O_3$	3,396.8	2,10	B. & A.
Do	$C_4H_8N_2O_3$	3,514.0	2,10	St. & L.
Do	$C_4H_8N_2O_3$	3,428.0	2,10	St.
Creatin cryst	$C_4H_9N_3O_2.H_2O$	3,714.1	1,2,10	St. & L.
Creatin, anhydrous	$C_4H_9N_3O_2$	4,275.4	1,2,10	St. & L.
Do		(3,206.0)	2	D.
Uric acid	$C_5H_4N_4O_3$	2,754.0	2,10	B. & M.
Do	$C_5H_4N_4O_3$	2,749.9	1,2,10	St. & L.
Do	$C_5H_4N_4O_3$	2,621.0	1,2,5,10	St.
Do	$C_5H_4N_4O_3$	2,645.0	1	Fr.

TABLE I.—*Heats of combustion of 1-gram substance, expressed in small calories*—Cont'd.

Substance.	Formula.	Calories.	References.	Investigators.
AMIDES, ETC.—continued.				
Guanin	$C_5H_5N_5O$	3,891.7	1,2	St. & L.
Guanidin	CH_5N_3	4,197.0	10	M.
Caffein	$C_8H_{10}N_4O_2$	5,231.4	1,2,10	St. & L.
FATS (ANIMAL).				
Fat of swine		9,476.9	2	St. & L.
Do		9,380.0	2,3	St.
Do		9,686.0	2	D.
Do		9,423.0	1,2	R.
Do		9,515.0	2	Gi.
Fats of oxen		9,485.7	2	St. & L.
Do		9,357.0	2,3	St.
Do		9,427.0	2	Gi.
Do		9,686.0	1	D.
Fats of sheep		9,493.6	2	St. & L.
Do		9,406.0	2,3	St.
Do		9,530.0	2	Gi.
Fat of horse		9,410.0	2	St.
Fat of dog		9,330.0	2	St.
Fat of goose		9,345.0	2	St.
Fat of duck		9,324.0	2	St.
Fat of man		9,398.0	2	St.
Sperm oil		10,001.0	2	Gi.
Butter fat		9,215.8	2	St. & L.
Do		9,192.0	2,3,10	St.
Do		9,185.0	2	Gi.
Do		9,231.3	8	
Do		9,179.0	1	St.
Do		9,230.0	10	St. & L.
Fat, mean (man, oxen, sheep)		9,365.0	10	St.
Fat, mean (dog, swine)		9,423.0	10	R.
Fat, mean (goose, duck)		9,500.0	10	St. & L.
Do		9,372.0	1	St.
FATS (VEGETABLE).				
Olive oil (expressed)		9,328.0	2,10	St.
Do		9,471.0	2	Gi.
Do		9,323.0	3	St.
Do		9,467.0	8	
Do		9,455.0	1	St.
Do		9,471.0	2	St.
Do		9,442.0	10	St.
Poppy-seed oil		9,489.0	2,10	St.
Rape-seed oil		9,489.0	2,10	St.
Do		9,619.0	2,10	St.
Do		9,621.9	10	M.
Ether extract—Various seeds		9,130.0 9,467.0	2	St.
CARBOHYDRATES, ETC.				
Arabinose	$C_5H_{10}O_5$	3,714.0	2,6,10	B. & M.
Do	$C_5H_{10}O_5$	3,722.0	8,2,6,10	St. & L.
Do	$C_5H_{10}O_5$	3,695.0	5,2,10,4	St.
Xylose	$C_5H_{10}O_5$	3,739.9	2,6,10	B. & M.
Do	$C_5H_{10}O_5$	3,746.0	2,8,6,10	St. & L.
Fucose	$C_6H_{12}O_5$	4,340.9	2,6,10	St. & L.
Rhamnose (water free)	$C_6H_{12}O_5$	4,379.3	2,6,10	St. & L.
Rhamnose (crystallized)	$C_6H_{12}O_5H_2O$	3,909.2	2,6,10	St. & L.
Sorbinose	$C_6H_{12}O_6$	3,714.5	2,6,10	St. & L.
Galactose	$C_6H_{12}O_6$	3,721.5	2,6,10	St. & L.
Do	$C_6H_{12}O_6$	3,659.0	2,4	St.
Dextrose (glucose)	$C_6H_{12}O_6$	3,762.0	2,4	B. & V.
Do	$C_6H_{12}O_6$	3,742.6	2,8,6,10	St. & L.
Do	$C_6H_{12}O_6$	3,692.0	2,4,5,10,1	St.
Do	$C_6H_{12}O_6$	3,754.0	2,10	Gi.
Do	$C_6H_{12}O_6$	3,939.0	1	Rch.
Do	$C_6H_{12}O_6$	3,760.0	6	B.
Dextrose (hydrated)	$C_6H_{12}O_6H_2O$	3,567.0	1	Rch.
Levulose (fructose)	$C_6H_{12}O_6$	3,755.0	2,8,6,10	St. & L.
Glucoheptose	$C_7H_{14}O_7$	3,732.8	2,10	B. & F.
Cane sugar (sucrose)	$C_{12}H_{22}O_{11}$	3,961.7	2,4,10,9	B. & V.
Do	$C_{12}H_{22}O_{11}$	3,955.2	8,2,6,9,10	St. & L.
Do	$C_{12}H_{22}O_{11}$	3,866.0	2,5,10	St.
Do	$C_{12}H_{22}O_{11}$	4,001.0	2,10	Ru.
Do	$C_{12}H_{22}O_{11}$	3,921.0	2,10	Gi.
Do	$C_{12}H_{22}O_{11}$	3,958.7	9	T.
Do	$C_{12}H_{22}O_{11}$	4,176.0	1	D.

TABLE I.—*Heats of combustion of 1-gram substance, expressed in small calories*—Cont'd.

Substance.	Formula.	Calories.	References.	Investigators.
CARBOHYDRATES, ETC.—continued.				
Cane sugar (sucrose)	$C_{12}H_{22}O_{11}$	4,173.0	1	Rch.
Do	$C_{12}H_{22}O_{11}$	3,959.0	1	St.
Do	$C_{12}H_{22}O_{11}$	3,908.0	4	St.
Milk sugar (lactose)	$C_{12}H_{22}O_{11}$	3,951.5	2,6,10	St. & L.
Do	$C_{12}H_{22}O_{11}$	3,877.0	1,2,4,5,10	St.
Do	$C_{12}H_{22}O_{11}$	4,162.0	1	Rch.
Do	$C_{12}H_{22}O_{11}$	3,920.0	10	Gi.
Milk sugar hydrated	$C_{12}H_{22}O_{11}H_2O$	3,777.1	2,10	B. & V.
Do	$C_{12}H_{22}O_{11}H_2O$	3,736.8	2,8,6,10	St. & L.
Do	$C_{12}H_{22}O_{11}H_2O$	3,663.0	2,4,10	St.
Do	$C_{12}H_{22}O_{11}H_2O$	3,710.0	2	Gi.
Do	$C_{12}H_{22}O_{11}H_2O$	3,945.0	1	Rch.
Do	$C_{12}H_{22}O_{11}H_2O$	3,667.0	1	St.
Do	$C_{12}H_{22}O_{11}H_2O$	3,772.2	4	B.
Do	$C_{12}H_{22}O_{11}H_2O$	3,721.8	6	Gi.
Do	$C_{12}H_{22}O_{11}H_2O$	3,724.0	10	Gi.
Maltose	$C_{12}H_{22}O_{11}$	3,949.3	2,8,6,10	St. & L.
Do	$C_{12}H_{22}O_{11}$	4,163.0	1	Rch.
Maltose hydrated	$C_{12}H_{22}O_{11}H_2O$	3,721.8	2,6,10	St. & L.
Do	$C_{12}H_{22}O_{11}H_2O$	3,932.0	1	Rch.
Trehalose	$C_{12}H_{22}O_{11}$	3,947.0	2,10,6	St. & L.
Trehalose hydrated	$C_{12}H_{22}O_{11}H_2O$	3,550.3	2,10,6	St. & L.
Raffinose (melitriose)	$C_{18}H_{32}O_{16}$	3,928.0	10	St.
Do	$C_{18}H_{32}O_{16}$	4,020.0	2,6,10	B. & M.
Do	$C_{18}H_{32}O_{16}$	4,020.8	2,6,10	St. & L.
Raffinose (melitriose) hydrated	$C_{18}H_{32}O_{16}{}^{5}(H_2O)$	3,400.2	2,6	St.
Do	$C_{18}H_{32}O_{16}{}^{5}(H_2O)$	3,399.1	10	St. & L.
Melitose	$C_{12}H_{22}O_{11}$	3,880.0	4	St.
Do	$C_{16}H_{32}O_{16}$	4,122.2	5	St.
Melezitose	$C_{18}H_{34}O_{17}$	3,913.7	2,6,10	St. & L.
Glycogen	$(C_6H_{10}O_5)n$	4,190.6	2,6	St. & L.
Cellulose	$(C_6H_{10}O_5)n$	4,200.0	2,4	B. & V.
Do	$(C_6H_{10}O_5)n$	4,185.4	2,6,10	St. & L.
Do	$(C_6H_{10}O_5)n$	4,146.0	1,2,4,10	St.
Do	$(C_6H_{10}O_5)n$	4,152.9	6	Go.
Do	$(C_6H_{10}O_5)n$	4,155.0	10	Go.
Do	$(C_6H_{10}O_5)n$	4,205.0	10	B. & V.
Dextrin (dextran)	$(C_6H_{10}O_5)n$	4,180.4	2,10	B. & V.
Do	$(C_6H_{10}O_5)n$	4,112.3	2,10,6	St. & L.
Starch	$(C_6H_{10}O_5)n$	4,228.0	2,4,10	B. & V.
Do	$(C_6H_{10}O_5)n$	4,182.5	2,6,10	St. & L.
Do	$(C_6H_{10}O_5)n$	4,123.0	2,4,10	St.
Do	$(C_6H_{10}O_5)n$	4,164.0	2,6,10	Gi.
Do	$(C_6H_{10}O_5)n$	4,479.0	1	Rch.
Do	$(C_6H_{10}O_5)n$	4,116.0	1	St.
Inulin	$(C_6H_{10}O_5)n$	4,187.1	2,10	B. & V.
Do	$(C_6H_{10}O_5)n$	4,133.5	2	St. & L.
Do	$(C_6H_{10}O_5)n$	4,070.0	2,4,10	St.
Do	$C_{36}H_{60}O_{31}$	4,133.5	6,10	St.
ALCOHOLS.				
Methyl alcohol (liquid)	CH_4O	5,260.7	5	
Do	CH_4O	5,307.1	10	F. & S.
Do	CH_4O	5,321.5	10	St. & L.
Methyl alcohol (gas)	CH_4O	5,693.7	10,5	Th.
Ethyl alcohol (liquid)	C_2H_6O	7,068.0	2,10	B. & M.
Do	C_2H_6O	7,183.6	3,10,1	F. & S.
Do	C_2H_6O	6,980.0	1	B.
Do	C_2H_6O	7,095.0	9	A.
Do	C_2H_6O	7,044.0	5	St.
Ethyl alcohol (gas)	C_2H_6O	7,402.2	10	Th.
Do	C_2H_6O	7,321.7	10	B. & M.
Do	C_2H_6O	7,394.1	5,7	
Propyl alcohol (liquid)	C_3H_8O	8,010.3	5	St.
Do	C_3H_8O	8,005.2	10	Lu.
Propyl alcohol (gas)	C_3H_8O	8,310.0	10	Th.
Do	C_3H_8O	7,301.7	5,7	
Amyl alcohol	$C_5H_{12}O$	8,958.6	10	F. & S.
Do	$C_5H_{12}O$	9,021.8	10	Lu.
Do	$C_5H_{12}O$	9,005.7	5	St.
Glycerin	$C_3H_8O_3$	(4,112.4)	2	St. & L.
Do	$C_3H_8O_3$	4,312.4	10	St. & L.
Do	$C_3H_8O_3$	4,317.0	2,10	St.
Do	$C_3H_8O_3$	4,305.0	1	St.
Do	$C_3H_8O_3$	4,265.2	10	Lu.
Erythrit	$C_4H_{10}O_4$	4,075.0	10	St.
Do	$C_4H_{10}O_4$	4,112.5	10	Lu.
Do	$C_4H_{10}O_4$	4,117.6	10	B. & M.
Do	$C_4H_{10}O_4$	4,131.3	10	St.&L.&K.
Do	$C_4H_{10}O_4$	4,132.3	10,6	St. & L.

TABLE I.—*Heats of combustion of 1-gram substance, expressed in small calories*—Cont'd.

Substance.	Formula.	Calories.	References.	Investigators.
ALCOHOLS—continued.				
Erythrit	$C_4H_{10}O_4$	4,072.7	5	St.
Mannite	$C_6H_{14}O_6$	4,001.2	2,4,10	B. & V.
Do	$C_6H_{14}O_6$	3,997.8	2,6,10	St. & L.
Do	$C_6H_{14}O_6$	3,908.0	2	St.
Do	$C_5H_{14}O_6$	3,959.0	2,6,10	Gi.
Do	$C_6H_{14}O_6$	3,939.0	10	St.
Do	$C_6H_{14}O_6$	3,936.6	5,10	St.
Arabit	$C_5H_{12}O_5$	4,024.6	6,10	St. & L.
Dulcit	$C_6H_{14}O_6$	3,975.9	6,10	St. & L.
Do	$C_6H_{14}O_6$	4,006.0	4,10	B. & V.
Do	$C_6H_{14}O_6$	3,905.9	5	St.
Quercite	$C_6H_{12}O_5$	4,293.6	6	St.
Do	$C_6H_{12}O_5$	4,329.0	6	B.
Inosite	$C_6H_{12}O_6$	3,679.6	6	St.
Do	$C_6H_{12}O_6$	3,695.1	6	B.
ACIDS.				
Formic acid (liquid)	CH_2O_2	1,271.2	5	St.
Do	CH_2O_2	1,366.8	10	J.
Do	CH_2O_2	1,347.8	10	B.
Formic acid (gas)	CH_2O_2	1,508.5	10	Th.
Acetic acid	$C_2H_4O_2$	3,490.4	2	B. & M.
Do	$C_2H_4O_2$	3,505.2	1,10	F. & S.
Do	$C_2H_4O_2$	3,498.3	7	
Do	$C_2H_4O_2$	3,553.2	5	St.
Do	$C_2H_4O_2$	3,480.0	10	J.
Do	$C_2H_4O_2$	3,555.0	10	St. & R.
Acetic acid (gas)	$C_2H_4O_2$	3,755.0	10	Th.
Propionic acid	$C_3H_6O_2$	4,968.2	5	St.
Butyric acid	$C_4H_8O_2$	5,936.9	5	St.
Do	$C_4H_8O_2$	5,647.0	1,10	F. & S.
Do	$C_4H_8O_2$	5,939.8	10	St. & Ro.
Oxybutyric acid	$C_4H_8O_3$	4,536.0	10	Lu.
Palmetic acid	$C_{16}H_{32}O_2$	9,352.9	2,10	St. & L.
Do	$C_{16}H_{32}O_2$	9,226.0	2,10	St.
Do	$C_{16}H_{32}O_2$	9,216.8	5	St.
Do	$C_{16}H_{32}O_2$	9,264.8	10	Lu.
Do	$C_{16}H_{32}O_2$	9,316.5	10	F. & S.
Stearic acid	$C_{18}H_{36}O_2$	9,429.0	2,10	St.
Do	$C_{18}H_{36}O_2$	9,716.5	1,10	F. & S.
Do	$C_{18}H_{36}O_2$	9,412.0	1	St.
Do	$C_{18}H_{36}O_2$	9,886.0	1	Rch.
Do	$C_{18}H_{36}O_2$	9,745.0	1	R.
Oleic acid	$C_{18}H_{34}O_2$	9,494.9	10	St. & L.
Malonic acid	$C_3H_4O_4$	1,998.2	2,10	B. & Lu.
Do	$C_3H_4O_4$	1,960.0	2	St.
Do	$C_3H_4O_4$	1,998.3	10	St. & L.
Succinic acid	$C_4H_6O_4$	3,006.2	2,10	B. & Lu.
Do	$C_4H_6O_4$	3,019.0	2	St.
Do	$C_4H_6O_4$	2,996.0	1	Rch.
Do	$C_4H_6O_4$	2,937.0	1	St.
Do	$C_4H_6O_4$	3,026.3	10	St. & L.
Tartaric acid	$C_4H_6O_6$	1,745.0	1,2	St.
Do	$C_4H_6O_6$	1,407.0	1	Rch.
Citric acid	$C_6H_8O_7$	2,477.9	2,10	B. & Lu.
Do	$C_6H_8O_7$	2,397.0	2,10	St.
Do	$C_6H_8O_7$	2,477.9	2,10	B. & Lu.
Citric acid, hydrated	$C_6H_8O_7H_2O$	2,250.4	10	Lu.
Oxalic acid	$C_2H_2O_4$	659.0	1	Rch.
Do	$C_2H_2O_4$	569.0	1	St.
Do	$C_2H_2O_4$	571.0	10	St.
Do	$C_2H_2O_4$	678.6	10	St. & L.
Do	$C_2H_2O_4$	672.5	10	J.
Benzoic acid	$C_7H_6O_2$	6,360.5	5	St.
Do	$C_7H_6O_2$	6,322.3	9	St. & L.
Do	$C_7H_6O_2$	6,322.1	9	B. & Lu.
Do	$C_7H_6O_2$	6,281.0	10	St.
Do	$C_7H_6O_2$	6,315.0	10	St. & Ro.
Do	$C_7H_6O_2$	6,345.0	10	B. & Re.
Do	$C_7H_6O_2$	(7,663.0)	5	St.
Salicylic acid	$C_7H_6O_3$	7,236.0	5	St.
Do	$C_7H_6O_3$	5,286.2	10	St. & L.
Do	$C_7H_6O_3$	5,326.0	10	B. & Re.
HYDROCARBONS, ETC.				
Methane	CH_4	13,063.0	3,10	F. & S.
Do	CH_4	13,218.9	5,7	Th.
Do	CH_4	13,243.7	10	Th.
Do	CH_4	13,275.0	10	B.

TABLE I.—*Heats of combustion of 1-gram substance, expressed in small calories*—Cont'd.

Substance.	Formula.	Calories.	References.	Investigators.
HYDROCARBONS, ETC.—continued.				
Ethane	C_2H_6	12,144.2	7,5	
Do	C_2H_6	12,346.7	10	Th.
Do	C_2H_6	12,991.7	10	B.
Propane	C_3H_8	12,027.3	10	Th.
Do	C_3H_8	12,543.0	10	B.
Do	C_3H_8	12,582.0	5,7	
Acetylene	C_2H_2	11,919.7	7	Th.
Do	C_2H_2	11,923.0	10	Th.
Do	C_2H_2	12,112.0	10	B.
Ethylene	C_2H_4	11,858.0	3,10	F. & S.
Do	C_2H_4	11,894.4	7,5	
Do	C_2H_4	11,883.6	10	Th.
Do	C_2H_4	12,154.0	10	B.
Propylene	C_3H_6	11,730.0	10	Th.
Do	C_3H_6	12,045.0	10	B.
Do	C_3H_6	11,717.0	5,7	
Benzol (benzene)	C_6H_6	9,949.0	10	B.
Do	C_6H_6	9,977.5	10	St. & L.
Do	C_6H_6	9,997.0	10	St., Ro. & He.
Benzol (gas)	C_6H_6	10,041.0	10	B. & Og.
Do	C_6H_6	10,096.0	10	St., Ro. & He.
Do	C_6H_6	10,247.4	10	Th.
Naphthalene	$C_{10}H_8$	9,628.0	9,10	St. & L.
Do	$C_{10}H_8$	9,618.7	10	St. & L.
Do	$C_{10}H_8$	9,664.0	10	B. & R.
Do	$C_{10}H_8$	9,700.0	10	B. & Lu.
Do	$C_{10}H_8$	9,718.0	10	B. & V.
Do	$C_{10}H_8$	9,773.0	10	Ru.
Camphor (inactive solid)	$C_{10}HO_{16}O$	9,298.7	10	Lu.
Do	$C_{10}HO_{16}O$	9,291.6	9	St.
Do	$C_{10}HO_{16}O$	9,288.0	9	B.

Nearly all of these results were obtained by burning the substance in oxygen gas in the bomb; but if we examine and compare the heat values obtained by different investigators with the same compound, and note how one author quotes the results of one, and another those of a second, investigator as being the correct ones, we reach but one conclusion, namely, that some one is wrong. Two different results can not both be correct. The differences are in many instances so great that they can not possibly be ascribed to impure substances or ordinary analytical errors due to drying, weighing, etc. But who will take the responsibility of saying that one investigator is right and another wrong? Or that so much of one man's work and so much of the work of some one else is correct? Each of the two men whose names appear most frequently (Stohmann and Berthelot) has a large following. Many will accept Stohmann's results before those of anyone else, and the same may be said of Berthelot; yet there is nothing which indicates that there was some particular fault in the operation of one distinct from the other, for in some cases Stohmann, and in others Berthelot, has the higher figures for the same substance.

Judging from the records of the different investigators, the most that can be said is that with a few substances we have the heats of combustion to within the limits of analytical error, but with the majority only approximately so, and with a few not even that.

Evidently there is need of a revision in the heat values of organic substances so that only the reliable, or at least nearly correct, values should find their way into the tables of text-books, etc., and the faulty ones be dropped. Until such a systematic checking up of the older results by means of the very best and most modern apparatus has been done, the bomb calorimeter should be used freely in all investigations where the heats of combustion play a part, and the values found by actual determinations should be preferred to those obtained or computed from general tables. The bomb thermometer may also to a limited extent serve as a standard with which other thermometers in use may be compared.

VARIATIONS IN DETERMINATIONS ALREADY ON RECORD.

In order to make these figures a little plainer and to put the matter in more compact form I have selected some of the more common compounds from the previous table, and, placing the highest reported heat of combustion of a substance at 100, have shown in the following table the difference between it and the lowest result obtained by some other investigator:

TABLE II.—*Difference between the highest and lowest results on some common compounds referred to in Table I.*

Substance.	Number of investigators.	Difference.	Substance.	Number of investigators.	Difference.
		Per cent.			*Per cent.*
Hydrogen	8	1.86	Cane sugar	9	6.42
Charcoal	4	4.53	Milk sugar	4	6.85
Sulphur	4	4.33	Milk sugar, hydrated	9	7.17
CO to CO_2	5	1.60	Maltose	2	5.13
Hemoglobin	5	1.09	Maltose, hydrated	2	5.35
Milk casein	8	4.10	Cullulose	6	1.40
Egg albumin	4	2.72	Starch	6	2.65
Blood fibrin	7	4.52	Inulin	3	2.75
Peptone	4	7.98	Methyl alcohol liquid	3	1.13
Chondrin	3	8.11	Ethyl alcohol liquid	5	2.83
Urea	6	16.53	Glycerin	3	1.20
Glycocoll	4	2.67	Mannite	5	2.33
Hippuric acid	3	.46	Acetic acid	6	2.11
Asparagin	3	3.34	Palmetic acid	5	1.46
Uric acid	4	4.83	Stearic acid	5	4.80
Fat of swine	5	3.16	Oxalic acid	5	16.08
Fat of oxen	4	3.40	Benzoic acid	6	1.25
Butterfat	6	.57	Methane	4	1.60
Olive oil	7	1.56	Benzol liquid	3	.48
Arabinose	3	.73	Benzol gas	3	2.02
Galactose	2	1.68	Naphthalene	6	1.58
Dextrose	6	1.86	Camphor	3	.12

In chemical-laboratory work, whether it be by advanced students or professional chemists, it is required that the work of the analyst shall be within certain limits of error, varying, naturally, with the nature of the substance analyzed and the method employed. No fixed rule can be laid down as to the limits of error allowed, but for most of the more common substances—such as oxides, carbonates, many ores and clays, etc.—some of which have many ingredients to be determined, the total of the determinations must come, barring exceptional cases, within, say, about 99.75 per cent and 100.5 per cent, in order to be accepted. The extent to which the results obtained by different analysts on a single ingredient are allowed to vary is very variable. In a determination like that of total nitrogen, for example, the substance containing 18 per cent nitrogen, two analysts should come not farther than about 0.05 or 0.06 per cent apart in the results obtained, except for special reasons. Such a variation as, for instance, between 17.97 per cent and 18.03 per cent nitrogen would be equal to 0.33 per cent difference between the analysts.

Of the 44 cases just cited in Table II the difference between the results obtained by different investigators is below 1 per cent for only five substances. With most of the substances the analysts are several points apart, and in urea and oxalic acid there are differences of over 16 per cent.

That two determinations by the same analyst agree when the same quantity of the sample is used, under exactly the same conditions and manipulations, is no guaranty that the method is perfect or the result correct. If, on the other hand, results agree when charges varying in weight have been used under somewhat varying conditions within the working limits of the method, we feel more sure about their being correct.

CAUSES OF DIFFERENCES IN HEAT DETERMINATIONS.

The method for the determination of heat values of substances can not be considered perfect until work can be done as accurately as is possible in ordinary chemical analyses at least.

From the heat values given we are led to question: What can have caused these differences in the past? What causes differences at present?

Following the directions given in Wiley's Principles and Practice of Agricultural Analysis, Volume III, a number of determinations had been made in this laboratory by means of the apparatus already described, using the commercial oxygen gas found on the market, put up in iron cylinders under very high pressure. The water equivalent of the whole bomb system had been determined by burning cellulose and cane sugar in oxygen under 20 atmospheres' pressure in the bomb, 4,185 calories per gram being the heat value used for the cellulose and 3,955.2 calories per gram for the sugar. The average of a large number of determinations had given the value as 430 grams, and since exactly 2,000 grams of water was used in all cases, the factor was 2,430.

Apparently there was no difficulty in obtaining concordant results when like charges were burned, but with varying charges the work was not satisfactory. The analytical error with a small charge may of course magnify the percentage error, but, excluding this possibility, it should be possible with a small charge to obtain results indentical with those obtained from a larger charge of the same material. To say that 4,000 to 5,000 calories should be generated in the bomb in order to give good results, and that with only 2,000 calories the work is not satisfactory, is to say that the entire method is unreliable.

IMPURE OXYGEN.

Some difficulties which were experienced in elementary carbon and hydrogen determinations in which the same oxygen was used led to the examination of the oxygen for impurities in the form of combustible gases. It was found beyond a doubt that such impurities were present in the oxygen in quantity sufficient to be reckoned with, and the magnitude of the error caused by them will be referred to further on.

Now arose the questions: To what extent do these combustible gases influence the working of the bomb calorimeter? Is the water value of the bomb, determined by the use of this impure oxygen, correct? Can a correction and the water value be worked out correctly?

TESTING OF OXYGEN.

In order to give the apparatus a severe test and to try to work out an answer to some of these questions, using the impure oxygen itself for the purpose, the following method was adopted: Cellulose was chosen as the chief substance to be burned, and sugar in the form of rock candy was also used. By burning small charges of pure cellulose absorption blocks in the bomb calorimeter, charged with oxygen at various pressures, it was hoped that any increase found with the greater pressure of oxygen would represent the total impurity in the additional amount of oxygen equivalent to the increased pressure, and that, having ascertained the correction for impurities, it could be applied and the water value of the bomb worked out without difficulty. Cellulose, although a very hygroscopic substance, and on that account difficult to handle, was chosen for this work because it ignites readily and burns quickly and completely in a comparatively small excess of oxygen. It represents the main class of substances whose heats of combustion are determined in our work, and its heat value is fairly well established. Sugar, on the other hand, is a substance which does not ignite readily and which burns more slowly, often leaving a trace of unburned carbon even with plenty of oxygen.

It was soon learned that the combustible gases in the oxygen were not oxidized completely, and perhaps never can be fully burned in a bomb calorimeter, but the tests were carried out as planned. The problem was not so simple as at first thought, but it was hoped that the oxidation of the gases referred to would be in proportion to the density of the gas mixture or the heat generated in the bomb. This is by no means a perfect method, but it may be a means of helping over the difficulty when no pure oxygen or other means for comparison are at hand.

One lot of oxygen (Oxygen I) was tested with both cellulose and sugar in the manner described. A second lot (Oxygen II) was tested only with cellulose. In every instance the cellulose was dried at between 103° and 105° C. to constant weight.

The effect of the impurities may be seen in Tables III to VII, where the corrected rises in temperature will indicate the differences due to more or less oxygen and to the amount of heat generated in the bomb. But before we discuss these results we may examine Table III, where we find the heats of combustion of the various substances worked out as ~~in~~ the course of ordinary routine work, using the water value 430 for the bomb system. This value plus the 2,000 grams of water used makes the factor for the bomb system plus water equal to 2,430 grams.

The column headed "Total computed calories" refers to all the heat generated in the bomb, and includes the heat represented by the substance and the iron wire burned, the nitric acid formed and dissolved, and the heat due to the electric current whenever it failed to ignite the substance instantly.

Varying amounts of oxygen will change the water value of the system; hence in all cases where more or less than 20 atmospheres oxygen was used the temperature rises have been corrected to 20 atmospheres oxygen pressure. For this bomb—of about 360 c. c. capacity—the correction for 1 atmosphere oxygen is ±0.0046 per cent of the observed rise in temperature. Thus, taking for example the first rise referred to in Table III, the rise due to the burning of 0.5092 gram sugar in 10 atmospheres oxygen, corrected to 20 atmospheres oxygen, will be:

	°C.
Observed rise in temperature	0.8488
Correction for 10 atmospheres oxygen	.00039
Corrected rise	.84841

The heat values accepted for the following substances, and used in the computations of the bomb water values given in Table III, are:

	Calories per gram.
Cellulose	4,185.0
Sugar	3,955.2
Naphthalene	9,628.0
Camphor	9,290.0
Benzoic acid	6,322.0

The water values of the bomb as found worked out in Table III have not been used in any computation, but they will indicate how widely the individual values vary from 430—the value used in the calculations of heats of combustion.

TABLE III.—*Water value of the bomb calorimeter and heat value per gram of substance, worked out by using the water value 430.*[a]

Substance.	Lot of oxygen used.	Oxygen pressure.	Weight of substance.	Total computed.	Rise corrected for oxygen, 20 atmospheres.	Bomb water value.	Calories per gram.
		Atmospheres.	*Grams.*	*Calories.*	°*C.*		
Sugar	I	10	0.5092	2,050.26	0.8484	416.6	3,976.0
		10	.5019	2,013.96	.8352	411.3	3,983.1
		24	.5054	2,038.32	.8493	400.1	4,005.4
		24	.5096	2,046.35	.8558	390.1	4,020.2
		24	1.0000	4,106.14	1.7037	410.0	3,986.6
Cellulose	I	10	.3799	1,617.47	.6671	424.6	4,194.4
		10	.3840	1,628.16	.6746	413.5	4,213.8
		10	.3711	1,571.58	.6484	423.8	4,193.3
		15	.3789	1,604.70	.6678	403.1	4,232.3
		20	.3800	1,609.68	.6734	390.4	4,255.2
		20	.3737	1,584.11	.6604	398.7	4,240.2
		20	.3714	1,574.21	.6558	400.4	4,237.2
		20	.6800	2,868.06	1.1896	410.9	4,218.3
Cellulose	II	10	.4358	1,802.34	.7381	442.0	4,164.2
		10	.4257	1,803.55	.7395	438.7	4,169.8
		24	.4253	1,824.37	.7489	435.9	4,174.5
		24	.4276	1,815.50	.7450	436.8	4,173.4
		24	.6675	2,817.39	1.1579	433.1	4,179.5
		24	1.0035	4,229.14	1.7492	417.7	4,206.4
Naphthalene	II	20	.5274	5,108.42	2.1111	419.8	9,668.9
		20	.5430	5,255.04	2.1785	412.2	9,699.3
Camphor	II	20	.4671	4,360.28	1.7985	424.4	9,311.6
		20	.5034	4,703.37	1.9409	423.3	9,315.8
Benzoic acid	II	20	.6986	4,438.67	1.8390	413.6	6,365.1
		20	.7027	4,473.37	1.8520	415.4	6,360.4

[a] No correction for impurities in oxygen is applied.

These determinations, too few in number for very good averages, were made now and then as other work permitted; hence they cover months of time. A hasty glance over these figures would be enough to condemn the apparatus as being useless for accurate work or the investigator as being unskilled or careless. Of course the analytical errors of the small charges will, as already said, be magnified; hence the duplicates may not always agree closely. Looking a little closer at the table, we notice that all the different groups—that is, those which were treated alike as to quantity of substance and the amount of oxygen—agree fairly well, thus proving that the differences between the groups are not due to careless manipulation. The reason for the disagreements must be sought for elsewhere.

But how are we to interpret the figures, which, in some cases, apparently contradict each other? In the column giving calories per gram we find in the case of cellulose an extreme difference of over 2 per cent in the heat of combustion. Further, we notice that 0.5 gram sugar burned in 10 atmospheres oxygen gave as high results as when 1 gram sugar was burned in 24 atmospheres oxygen. Again, in the case of cellulose in Oxygen I, the smaller charges at 20 atmospheres oxygen gave higher results than the larger charge at the same oxygen pressure, whereas in the case of Oxygen II the opposite is shown.

Another very marked peculiarity in the results is that, with the same factor for the bomb, etc., and the same kind of cellulose, in the work with Oxygen I, all the results on both sugar and cellulose are above the accepted values—sugar as much as 1.6 per cent and cellulose up to 1.64 per cent. In Oxygen II, on the other hand, all the cellulose results, except the one where over 1 gram cellulose charge was used, are below the accepted heat value; but the naphthalene, camphor, and benzoic acid are all above—naphthalene about 0.7 per cent, camphor about 0.3 per cent, and benzoic acid about 0.7 per cent. It should be mentioned here, however, that these substances, while bought as chemically pure, were not tested for impurities before using.

Looking at the column giving the water values of the bomb, we find a corresponding variation, most of the values being below the one used (430), ranging anywhere from 390 to 442.

If a general view is taken of the various results and the behavior of the substances under different conditions, we are convinced that the oxygen contains considerable impurity in the form of combustible gases, for which corrections must be applied. This being the case, the old, apparently too high, water value (430) of the bomb will not be high enough.

CORRECTION FOR IMPURITY IN THE OXYGEN.

Is the correction a constant quantity? Is it of any value to know the total amount of carbon and hydrogen present as combustible gases in the oxygen? These are some of the questions which now concern us as we endeavor to work out the water value of the bomb system, applying the corrections as found for the oxygen and for the amount of heat generated in the bomb.

We turn our attention to Tables IV to VII and examine first the work with cellulose, Oxygen I. From Table IV we learn that between the averages computed to be due to like charges burned in ten atmospheres oxygen pressure and 20 atmospheres there is a slight difference in the rise in temperature in favor of the greater amount of oxygen. This difference does not represent the total impurity in that amount of oxygen, but only the amount which will be oxidized under the specific conditions of so much cellulose and so much oxygen. The difference in rise in temperature, when about 1,600 calories were generated in the bomb, was 0.000665° C. for 1 atmosphere oxygen.

TABLE IV.—*Rise in temperature caused by burning like quantities of cellulose in the bomb calorimeter, in varying amounts of oxygen (Oxygen I).*

Oxygen pressure.	Cellulose.	Fuse wire.	HNO_3.	Total computed.	Rise in temperature.	Rise corrected for oxygen, 20 atmospheres.	Rise due to 1,600 calories.
Atmospheres.	*Grams.*	*Calories.*	*Calories.*	*Calories.*	° *C.*	° *C.*	° *C.*
10	0.3799	17.12	2.60	1,617.47	0.6674	0.6671	0.6599
10	.3840	17.12	4.00	1,628.16	.6749	.6746	.6629
10	.3711	16.48	2.00	1,571.58	.6487	.6484	.6601
15	.3789	16.00	3.00	1,604.70	.6679	.6678	.6658
20	.3800	15.68	3.70	1,609.68	.6734	.6734	.6694
20	.3737	15.68	4.50	1,584.11	.6604	.6604	.6670
20	.3714	16.00	3.90	1,574.21	.6558	.6558	.6665

	°C. rise.
1,600 calories in 20 atmospheres oxygen pressure	0.66763
1,600 calories in 10 atmospheres oxygen pressure	.66098
Difference	.00665
Rise per atmosphere oxygen pressure	.000665

Whether this error is uniform throughout—that is, whether the eleventh atmosphere oxygen, for instance, would show the same error as the twenty-first atmosphere with the same charge—can not be ascertained from these figures. At present we can only assume the error to be uniform.

Is this then the true correction to apply, no matter how large a charge was burned, or will more of the gases be oxidized in the same quantity of oxygen when more heat is generated?

Rock candy, Oxygen I.—In Table V we find a difference in the rise of temperature between 10 and 24 atmospheres oxygen pressure equal to 0.00045° C. per atmosphere when about 2,000 calories are generated in the bomb.

Table V.—*Rise in temperature caused by burning like quantities of rock candy in the bomb calorimeter in varying amounts of oxygen (Oxygen I).*

Oxygen pressure.	Rock candy.	Fuse wire.	HNO_3.	Total computed.	Rise in temperature.	Rise corrected for oxygen, 20 atmospheres.	Rise due to 2,000 calories.
Atmospheres.	*Grams.*	*Calories.*	*Calories.*	*Calories.*	°*C.*	°*C.*	°*C.*
10	0.5092	18.08	3.25	2,050.26	0.8488	0.8484	0.8276
10	.5019	16.80	3.15	2,013.96	.8356	.8352	.8293
24	.5054	19.52	4.10	2,038.32	.8491	.8493	.8333
24	.5096	13.16	4.50	2,046.35	.8556	.8558	.8364

	°C. rise.
2,000 calories in 24 atmospheres oxygen pressure	0.83481
2,000 calories in 10 atmospheres oxygen pressure	.82847
Difference	.00634
Rise per atmosphere oxygen pressure	.00045

Sugar, as has been said, does not always burn completely. Frequently a hardly weighable amount of carbon may be seen on the platinum capsule, a very small spot perhaps, yet it is an indication of incomplete combustion of the carbon itself and of the possibility that at least traces of intermediate partially oxidized products may be found.

Cellulose, Oxygen II.—In Table VI we find the results obtained with cellulose and another lot of oxygen. Here 1,800 calories produced in oxygen at 10 and 24 atmospheres pressure, a difference in temperature of only 0.00007° C. per atmosphere, which is within the limits of analytical error.

TABLE VI.—*Rise in temperature caused by burning like quantities of cellulose in the bomb calorimeter in varying amounts of oxygen (Oxygen II).*

Oxygen pressure.	Cellulose.	Fuse wire.	HNO_3.	Total computed.	Rise in temperature.	Rise corrected for oxygen, 20 atmospheres.	Rise due to 1,800 calories.
Atmospheres.	*Grams.*	*Calories.*	*Calories.*	*Calories.*	°*C.*	°*C.*	°*C.*
10	0.4258	17.12	3.25	1,802.34	0.7384	0.7381	0.7371
10	.4257	19.20	2.80	1,803.55	.7404	.7395	.7386
24	.4253	21.12	5.00	1,824.37	.7488	.7489	.7389
24	.4276	21.44	4.55	1,815.50	.7449	.7450	.7387

	° C. rise.
1,800 calories in 24 atmospheres oxygen pressure	0.73881
1,800 calories in 10 atmospheres oxygen pressure	.73778
Difference	.00103
Rise per atmosphere oxygen pressure	.00007

We have reason to believe, however, judging from the determinations referred to in Table III that this oxygen supply also was not pure, and hence we take up the question asked before: What effect has increased heat production upon the oxidation of these combustible gases?

For some of the following comparisons I must depend upon single determinations, which in itself is not very satisfactory; but since no more of the same oxygen is at hand for further work I must take for granted that the determinations are correct.

TABLE VII.—*Comparison of observed rise in temperature due to the large and the calculated rise due to the smaller charges of a substance burned in the same quantity of oxygen.*

Substance.	Lot of oxygen used.	Oxygen pressure.	Weight of substance.	Computed total.	Rise.	
		Atmospheres.	*Grams.*	*Calories.*	°*C.*	
Sugar	I	24	1.0000	4,106.14	1.70366	Observed.
		24	.5075	2,042.33	1.71396	Calculated.
				2,063.81	−.01020	Difference.
Cellulose	I	20	.6800	2,868.06	1.1896	Observed.
		20	.3750	1,589.30	1.1967	Calculated.
				1,278.76	−.0071	Difference.
Cellulose	II	24	1.0035	4,229.14	1.7492	Observed.
		24	.4265	1,819.93	1.7359	Calculated.
				2,409.21	+.0133	Difference.
		24	.6675	2,817.39	1.1579	Observed.
		24	.4265	1,819.93	1.1564	Calculated.
				997.46	+.0015	Difference.

In Table VII we find that when 1 gram of sugar was burned in 24 atmospheres oxygen, 4,106.14 calories of total heat being generated in the bomb, the rise in temperature of the bomb system was found to be 1.70366° C. The average rise in temperature found when 0.5075 gram of sugar was burned, or 2,042.33 calories of total heat was generated in the bomb, in the presence of 24 atmospheres oxygen, was 0.8525° C., and according to that the generation of 4,106.14 calories should have caused a rise of 1.71396° C. instead of 1.70366° C., as found when 1 gram was burned. The difference, therefore, is a minus of 0.0102° C. This indicates that, of the impurities in this particular oxygen, more were oxidized in proportion as the charge of sugar was reduced. Hence the correction referred to in Table V should not be increased in proportion to the increase in the amount of substance used.

With cellulose in Oxygen I a charge of 0.6800 gram, which, plus wire, etc., was equivalent to 2,868.06 calories total heat generated, gave a rise of 1.1896° C. instead of 1.1967° C. as it should have done according to the results upon the smaller charges. Here is also a minus difference of 0.0071° C., showing that this oxygen behaved the same with these two different substances. The combustible gases present in the oxygen must accordingly be of an easily combustible nature, comparatively speaking, when the increase in heat has no effect upon them above that of the lesser heat.

Next we examine the results obtained with different amounts of cellulose in Oxygen II. Here we see not a decrease, but a very marked increase in the oxidation of combustible gases with increase in heat evolved. A charge of 0.6675 gram cellulose, or a total heat production of 2,817.39 calories, gave a rise in temperature of 1.1579° C., which is 0.0015° C. above what it should have been according to calculation from the results of the smaller charge of 0.4265 gram cellulose, equal to 1,819.93 calories total heat. When 1.0035 grams cellulose was burned there was a difference of 0.0133° C. above the calculated result. This difference in behavior of the two oxygen supplies toward larger charges of the substances burned indicated a difference in composition of the combustible gases in the oxygen. In Oxygen I the gases were apparently more readily oxidized than in Oxygen II, and therefore more of them burned with the small charges in proportion to what burned with the larger. The opposite would then be true of Oxygen II. Unfortunately no further and more definite proof can be given, since no qualitative tests were made of the gases. As to the impurities in Oxygen I being different from those in Oxygen II, there can be no doubt. In the first lot a strong, peculiar, disagreeable odor was noticed, and when the gases passed through pumice stones saturated with H_2SO_4 a slight yellow coloration was noticed for a short distance. Oxygen II had no such disagreeable odor and did not color the pumice stone as did Oxygen I.

According to analyses made by one of the assistant chemists of the experiment station, Oxygen I contained 0.0355 per cent of hydrogen and 0.0150 per cent of carbon by weight, while Oxygen II contained 0.0335 per cent of hydrogen and 0.0150 per cent of carbon.

These results show that there must have been considerable free hydrogen present.

Not knowing the composition of the gases referred to, we may, for the sake of obtaining at least an approximate calorific value, assume that all the carbon is present as marsh gas, and that the excess of hydrogen is free hydrogen. Thus we have in Oxygen I 0.02004 per cent CH_4 and 0.01446 per cent free H_2, and in Oxygen II 0.02004 per cent CH_4, and 0.01346 per cent free H_2.

With 20 atmospheres oxygen, or in round numbers 10 grams of oxygen, these gases would represent in Oxygen I 73 calories, equivalent to 0.0299° C. rise, and in Oxygen II 69 calories, equivalent to 0.0283° C. rise.

In none of the determinations referred to with the above-mentioned oxygen was more than about 45 per cent of these figures reached, which can be seen in Table IV, where the correction for the impurities in 20 atmospheres oxygen equals 0.0133° C. This indicates plainly that only a part of the combustible gases mixed with the oxygen can be oxidized in the bomb.

RISE IN TEMPERATURE CORRECTED FOR HEAT DUE TO IMPURITIES IN THE OXYGEN.

In Table VIII following are given the corrected figures for the rise in temperature, also the values computed for the bomb water value and for the heats of combustion of the substances, respectively. The corrected figures for the rise in temperature are obtained by using the results of Tables IV to VI. Thus the corrections used for the sugar and cellulose burned in Oxygen I are the differences found in Tables IV and V. No account has been taken of any increase in total heat formed in the bomb by use of larger amounts of material with Oxygen I.

For the smaller charges of cellulose burned in Oxygen II the differences found in Table VI have been used, and for the larger charges the increase in rise of temperature actually observed (Table VII) has been added to the first-mentioned correction. Thus, where 0.4253 gram cellulose was burned in Oxygen II the correction will be $0.00007° \times 24 = 0.00168°$ C. For the charge of 0.6675 gram cellulose the correction will be $0.00168° + 0.0015°$ C., and for the largest amount of cellulose burned the correction equals 0.0151° C.

To all determinations of the other substances—naphthalene, camphor, and benzoic acid—where the total heat was a little higher than it was with the cellulose charge last mentioned, the same correction, 0.0151° C., has been applied.

The heats of combustion were computed by using the bomb water value 439.2. This value is the average of those found for cellulose, naphthalene, camphor, and benzoic acid with Oxygen II. The average of all the results in Table VIII would be 437.8, but the values obtained with Oxygen I were not used on account of the sugar and cellulose not agreeing very closely.

TABLE VIII.—*Water value of the bomb calorimeter, and heat value per gram substance, worked out by using the new water value, 439.2, found after applying the corrections for the impurities in the oxygen.*

Substance.	Lot of oxygen used.	Oxygen pressure.	Weight of substance.	Total computed.	Rise corrected for oxygen per 20 atmospheres.	Bomb water value.	Calories per gram.
		Atmospheres.	*Grams.*	*Calories.*	°*C.*		
Sugar	I	10	0.5092	2,050.26	0.8439	429.5	3,969.7
		10	.5019	2,013.96	.8307	424.4	3,976.4
		24	.5054	2,038.32	.8385	430.9	3,969.0
		24	.5096	2.046.35	.8450	421.7	3,984.2
		24	1.0000	4,106.16	1.7031	411.0	4,001.7
Cellulose	I	10	.3799	1,617.47	.6605	449.0	4,188.9
		10	.3840	1,628.16	.6678	438.1	4,186.9
		10	.3711	1,571.58	.6418	448.7	4,168.7
		15	.3799	1,604.70	.6578	439.5	4,184.8
		20	.3800	1,609.68	.6601	438.6	4,158.6
		20	.3737	1,584.11	.6471	448.0	4,170.1
		20	.3714	1,574.21	.6425	450.2	4,166.4
		20	.6800	2,868.06	1.1834	423.6	4,212.6
Cellulose	II	10	.4258	1,802.34	.7373	444.5	4,178.0
		10	.4257	1,803.55	.7388	441.2	4,181.5
		24	.4253	1,824.37	.7472	441.7	4,180.5
		24	.4276	1,815.50	.7433	442.5	4,179.0
		24	.6675	2,817.39	1.1548	439.7	4,184.1
		24	1.0035	4,229.14	1.7341	438.8	4,185.7
Naphthalene	II	20	.5274	5,108.42	2.0960	437.2	9,635.6
		20	.5430	5,255.04	2.1634	429.1	9,668.3
Camphor	II	20	.4671	4,360.28	1.7834	444.9	9,268.1
		20	.5034	4,703.87	1.9258	442.3	9,277.9
Benzoic acid	II	20	.6986	4,438.67	1.8239	433.6	6,336.6
		20	.7027	4,473.37	1.8369	435.3	6,332.1

The corrected results in Table VIII agree fairly well among themselves, and even where the conditions for determinations differed so widely from the ordinary they approach the heat values generally accepted for the substances used. This certainly could not be said when the impurities were not considered, as was seen in Table III, where a higher value was used than the average of the water values given in that table. From all that has been said on this topic we learn that, at best, the determination of the hydrothermo equivalent, or water value, of the bomb with impure oxygen is no easy task, nor can it be very satisfactory. Hence the method explained can be recommended for use only where no pure oxygen—i. e., oxygen free from combustible gases—can be obtained.

FORMATION OF NITRIC ACID IN THE BOMB CALORIMETER DURING COMBUSTION.

The formation of HNO_3 in the bomb has already been alluded to, also that correction must be made for the heat represented by the HNO_3 solution.

In the bomb there is always present more or less free nitrogen, and a portion of it is always oxidized, varying in quantity according to the nature of the substance burned, the total heat generated, and the quantity of nitrogen in the bomb. This quantity of HNO_3, which appears to be constant for specific conditions, I propose to designate, for convenience, "bomb nitric acid," meaning thereby the HNO_3, expressed in calories, which would be formed in the bomb from free nitrogen during the combustion of a specified substance under specified conditions. Table IX, following, gives us an idea as to the value of this quantity for the oxygen used. This quantity, however, represents only a very small portion of the free nitrogen present in the bomb.

TABLE IX.—*Formation of HNO_3 in the bomb calorimeter during combustion of nonnitrogenous substances.*

Substance.	Determinations.	Oxygen.	Weight of substance.	Total heat.	HNO_3.	HNO_3 per 1,000 calories.
	Number.	*Atmospheres.*	*Grams.*	*Calories.*	*Calories.*	*Calories.*
Cellulose	6	24	0.5481	2,335.2	6.74	2.89
Do	7	20	.6550	2,763.9	6.50	2.35
Do	6	10	.3946	1,677.4	3.01	1.79
Sugar	4	24	.7538	3,016.4	6.13	2.04
Do	7	20	1.2437	5,019.7	9.87	1.97
Do	2	10	.5056	2,019.0	3.20	1.58
Naphthalene	2	20	.5352	5,182.2	8.82	1.70
Camphor	2	20	.4853	4,531.9	7.70	1.70
Benzoic acid	2	20	.7007	4,454.6	7.55	1.70

In the last column of the foregoing table we see that this indifferent gas nitrogen behaves very much like the combustible gases present as impurities in the oxygen, namely, with an increase in quantity of oxygen, accompanied by an increase of free nitrogen, more nitrogen was oxidized. The individual determinations also showed a tendency to increased nitrogen oxidation with an increase in amount of substance burned.

For cellulose and the class of substances which it represents, such as feeds and feces, we can use, without introducing any appreciable error in the following calculations, a correction of 2.4 calories for each 1,000 calories total heat at 20 atmospheres oxygen pressure for the HNO_3 formed. For sugar and substances which would burn more like sugar than cellulose, such as gelatin, dried urine, milk, etc., we may use 2 calories per 1,000 calories total heat as the corresponding correction for the HNO_3 formed.

OXIDATION OF COMBINED NITROGEN TO NITRIC ACID.

It is generally assumed that the combined nitrogen in organic substances is set free as elementary nitrogen during a combustion. But in view of what has already been said we may be led to question whether all, or at least part, of this nitrogen may not also be burned to HNO_3, perhaps even more readily so than the free nitrogen gas. It will be my object to take up the question of nitrogen of the substances to be analyzed, to see how much of this nitrogen is oxidized during a combustion, and what effect, if any, this will have upon the general results.

To illustrate the points which I want to bring out in this connection, and to make the tabulated figures more reliable, I have collected and taken the average of a large number of figures from work done at this station by Messrs. Norris and Carpenter on feeds, excreta, etc., the heats of combustion of which were mostly used in connection with the nutrition investigation experiments with cattle by means of the respiration calorimeter, referred to earlier in this paper. These data have served as the chief basis for the computations set forth in Table X. The substance in each charge was either a nitrogenous compound or mixed with one, so that in each case nitrogen was present in a combined form. From such a variety of substances we may expect to find some differences brought out as to the behavior in regard to the nitrogen, if any difference exists.

TABLE X.—*Average HNO_3 formed during the combustion of various nitrogenous substances in the bomb calorimeter.*

Substance.	Single determinations.	Average charge.	Total heat.	Nitrogen in charge.	Bomb HNO_3.	HNO_3 found.
	Number.	*Grams.*	*Calories.*	*Grams.*	*Calories.*	*Calories.*
Clover hay	79	1.0178	4,355.8	.0183	10.45	5.68
Grain feed	16	1.0031	4,508.9	.0342	10.82	9.51
Corn meal	18	1.0046	4,252.9	.0167	10.21	9.38
Linseed meal	11	1.0110	4,923.4	.0616	11.80	13.92
Feces	70	1.0144	4,454.3	.0191	10.69	10.33
Hair and dandruff	17	.8218	3,828.0	.0601	7.66	34.10
Gelatin	5	.6601	2,671.4	.1188	5.34	12.42
Alcohol+ Gelatin	16	.6819 .2006	4,392.0 965.6	.0349	12.86	17.81
Urine+ Much cellulose	20	4.7571 .6623	1,182.8 2,771.7	.0462	7.91	.00
Urine+ Little cellulose	29	7.5178 .0508	1,548.3 212.8	.0478	3.52	1.78
Milk+ Cellulose	9	1.4648 .6596	1,103.9 2,760.4	.0079	9.27	9.22
Milk alone	9	4.0354	3,327.9	.0225	6.66	9.17

In the above table we find, besides the amount of the sample used and the total heat produced in the bomb, the total nitrogen in the charge taken, the bomb nitric acid as calculated by using the values obtained in Table IX, and the actual HNO_3 found. It was assumed that the acidity in the bomb was due entirely to HNO_3. The figures represent the averages of numerous closely agreeing determinations; in the case of hay, 79 single determinations. It may be stated here that the nitrogen represented by 1 calory is equal to 0.00098 gram, or to 0.004406 gram HNO_3.

From these figures we learn first of all that the nitrogen behaves differently in different substances. Hair and dandruff, for instance, gave 4.5 times as much nitric acid as was due to the oxidation of the atmospheric nitrogen in the bomb, whereas gelatin, which contained about twice as much total nitrogen in the sample, gave not much more than one-third as much nitric acid as did the hair and dandruff, and only 2.3 times as much as the bomb nitric acid called for. In the case of urine plus considerable cellulose there was, strangely enough, no nitric acid found at all, yet about 8 calories should have been formed from the free nitrogen of the bomb alone. But with urine plus a little cellulose a small amount of HNO_3 was found. Naturally, one would expect more where more cellulose was used. These variations may introduce errors into the computations of the heat values of the substances, and in order to see the extent of these variations more clearly, and perhaps the probable errors, I have presented the results in another form in Table XI.

TABLE XI.—*Errors due to the oxidation of nitrogen.*

Substance.	Per cent found of total HNO_3 possible.	Nitrogen of sample oxidized to HNO_3.	±Deviations from bomb HNO_3.	Errors of total calories of sample.
		Per cent.	*Calories.*	*Per cent.*
Clover hay	19.47	0.0	− 4.77	0.11
Grain feed	20.80	.0	− 1.31	.03
Corn meal	34.37	.0	− .83	.02
Linseed meal	18.61	3.37	+ 2.12	.04
Feces	34.18	.0	− .36	.01
Hair and dandruff	49.56	43.02	+26.44	.70
Gelatin	9.80	5.83	+ 7.08	.27
Alcohol+gelatin	46.97	19.75	+ 4.95	.11
Urine+much cellulose	.0	.0	− 7.91	.69
Urine+little cellulose	3.40	.0	− 1.74	.11
Milk+cellulose	56.39	.0	− .05	.00
Milk alone	30.91	10.91	+ 2.51	.08

The bomb nitric acid plus the nitric acid represented by the oxidation of the total nitrogen of the charge is called 100, and in the first column of this table are found, expressed in percentages, the amounts formed of this possible HNO_3. In the second column is given the percentage of the nitrogen of the sample oxidized to HNO_3. The last two columns give the variations of nitric acid found from the bomb HNO_3, and the percentage of the total heat values represented by this number of calories, as calculated from the determinations.

Several plus and several minus deviations from the computed bomb nitric acid are noticed in the preceding table. The minus signs force us to question: Was the full amount of bomb HNO_3 not formed, or was it formed and did it disappear again, changed into some other compound? The work done is not enough to answer this question fully, but I am strongly inclined to believe that the nitric acid was formed, but immediately on forming was changed into some other combination, probably through the influence of the ash ingredients.

In the bomb the nitric acid is found dissolved in the moisture which is condensed on the sides of the bomb. Here it may be mentioned that in transferring the nitric acid from the bomb to a beaker for titration care should be taken not to wash out the platinum capsule or add the ash to the rinsing. Often the ash is very strongly alkaline and would make the whole solution alkaline, which would be the same as having a minus quantity of acid formed—an impossibility.

This finding of the nitric acid and water together on the sides of the bomb would seem to indicate that the nitric acid is formed as a gas, and, together with the water in gaseous form, is expelled or driven from the seat of the great chemical activity or point of combustion to unite and condense with the moisture on the cool sides of the bomb. But this can not always be the nature of the combustion, judging from the sample of urine plus considerable cellulose. Here also the water was condensed on the sides of the bomb, and from the cellulose alone there should have been several calories HNO_3 dissolved in it, but the water was neutral. The ash, on the other hand, was decidedly alkaline. Where the urine was burned with but a small quantity of cellulose one-half of the bomb HNO_3 was found in the water. Here, too, the ash was alkaline. Nitric acid, it seems, can not be formed by the action of the flame and heat on nitrogen away from the ash or skeleton of the substance burned, but must be formed in the capsules holding the substance at the very point where the heat first attacks the compound, the place of greatest chemical activity. We can imagine that in a remarkably short time numberless chemical changes must take place at that point. The nitric acid does not leave this seat of action when there is some attraction strong enough to hold it. In the case of urine and other substances such an attraction seems to be in the ash, and hence little or none of the nitric acid escapes with the other gases formed to the sides of the bomb. The attraction referred to can hardly be anything but the chemical affinity of the alkalies of the ash, which unite with the acids formed, thus holding at least part if not all of them. In this we have an explanation of how it is possible to have less than the bomb HNO_3, since ash with alkaline reaction will prevent nitric acid from being dissolved in the water and perchance prevent in part its very formation.

Eight ashes left by the combustion of urine plus a little cellulose were analyzed for nitrogen, and the average of the results gave the equivalent of 1.1 calory HNO_3, which represents most of the 1.74 calories of missing nitric acid, as shown in Table X. Unfortunately, only these 8 ashes were saved for nitrogen determination. What the nitrogen compounds were in the ash was not determined, nor do we know anything about what part of the missing nitric acid was present in the ash, in the case of the urines, where all was missing, none being dissolved in the water in the bomb.

PROBABLE ERROR DUE TO DISAPPEARANCE OF NITRIC ACID.

Taking for granted that the plus values in the table refer to the total nitric acid formed, and that not any of it has united with anything else, the plus then does not mean an error, since all the nitric acid formed is measured and accounted for in the calculations. But the minus or missing nitric acid is in all probability always an error which would be greater or less than the values given in the last column, according to the nature of the combination formed. Thus errors are liable to be introduced when, in the operation of the bomb and computations of the results, the acidity in the solution titrated against NaOH solution is assumed to be HNO_3, and therefore the amount of heat represented by it always subtracted from the total heat generated in the bomb.

There are other acids formed in the bomb besides nitric acid, and directions for the manipulation of the bomb calorimeter should make specific mention of this fact and explain how they should be considered, so as not to introduce any error. They are not in any way equivalent to HNO_3, and should therefore not be treated as such. The writer has in mind especially the H_2SO_4.

It is generally accepted that in a complete combustion of a nitrogenous organic substance the nitrogen returns to free nitrogen and oxidizes only under extraordinary conditions such as may be produced by a powerful electric spark, or which exists in the bomb calorimeter where the combustion takes place in the presence of large excess of oxygen, etc. For this reason any heat caused by the formation of HNO_3 must be subtracted from the total heat generated in the bomb.

With sulphur compounds it is different. The natural combustion product of sulphur is the acid, and hence any heat produced by the formation of H_2SO_4 belongs to the compound and should not be subtracted. Especially is this true when the bomb results are to be compared with the combustions of organic substances as they take place in the animal body. Part or all of the sulphuric acid may be held in the form of salts in the ash, but as the ash is practically an unexplored field, it will not be touched upon at all at this time.

Whether the H_2SO_4 is counted in as HNO_3 or not will at times make a great difference in the final results. This may be seen very clearly from some work which the writer did upon dandruff and hair (brushed off a steer at the daily cleaning), upon pure hair, and upon two samples of steer urine.

The results so far as they bear upon this point are found in Table XII.

TABLE XII.—*Difference in the heat values found when the total acidity is regarded as HNO_3 and when the H_2SO_4 has been subtracted.*

Substance.	Charge.	Acid solution titrated against NaOH solution.			Calories per gram substance.		Difference.
		Total alkali.	Due to H_2SO_4.	Difference HNO_3.	Acidity called HNO_3.	Titration corrected for H_2SO_4.	
	Grams.	*c. c.*	*c. c.*	*Calories.*			*Per cent.*
Brushings (dandruff and hair)	1.0236	68.94	62.98	5.96	4,052.4	4,113.0	1.48
Do	.8959	59.79	58.88	.91	4,048.7	4,125.7	1.87
Do	.8809	59.62	58.27	1.35	4,066.6	4,132.7	1.60
Hair	.9133	49.94	38 54	11.40	5,180.2	5,221.4	.79
Urine	7.2462	1.60	1.1	3.97	153.36	153.42	.04
Do	6.1216	1.65					
Do	6.0444	1.82					
Do	7.6021	2.92	3.1	6.37	175.6	175.73	.07
Do	7.8134	3.19					
Do	8.4447	3.36					

These few determinations will suffice to show that a very large error may be introduced by the H_2SO_4. In the case of urine the error introduced by reckoning sulphuric acid as nitric acid is small, but with the hair it reached about 0.8 per cent, and in the case of the epidermic tissue, dandruff, etc., which is richer in sulphur, the error was in one instance about 1.9 per cent.

CAUSE OF INCOMPLETE COMBUSTION.

The shape and size of crucible, or capsule, in which the substance is burned may influence the combustion.

Owing to inability to watch the process of the combustion in the bomb, the general opinion, gained perhaps from the observations of explosives, is that the combustion in the bomb calorimeter is very sudden and violent, in nature like an explosion in the free air. This, however, is not correct. The time of combustion varies with the nature of the substance, but, with the materials mentioned in the previous tables, only by a very few seconds. It is of a very short duration, but by no means an instantaneous flash. The supposed violence, implying that the flash fills the bomb, has absolutely no foundation and would not happen except when the oxygen contains a considerable percentage of highly combustible gases. To prove the correctness of this statement direct tests were made as follows: Small pieces of filter paper, about one-fourth inch square, were fastened by one edge to the sides, top, and bottom of the bomb by means of paste, and allowed to dry. In one trial the burning of a small charge of cellulose did not affect the few pieces of filter paper which were fastened on the top and on the bottom of the bomb. After that a more complete test was made by fastening three pieces to the top, one at the center of the bottom, and ten distributed on the sides of the bomb; four of them about opposite the top of the capsule, four nearer the top, and two nearer the bottom. The charge was 1 gram powdered rock candy plus a little naphthalene, burned in 20 atmospheres oxygen. The test resulted in two of the three pieces on the top being burned, the third one showing in one corner a trace of brown as when paper has been held near strong heat. All the rest were absolutely untouched. Hence, the flames may at times reach the top, and perhaps more seldom the sides, though the distance is much shorter. With more oxygen the flame very likely would not even have reached the top of the bomb.

It is a known fact that fire will put itself out in a closed room, even before the oxygen is fully consumed. If this is true in a room, might it not also be true in a deep capsule surrounded by plenty of oxygen? To have some light shed upon this question, and to be able to decide in favor of some one of the different shaped capsules used, the following tests were made: A wide-mouthed bottle of 1,500 c. c. capacity was filled with oxygen, and the crucible, attached to a wire fastened in the cork, was let down in the bottle, and the substance ignited by means of an iron fuse wire as in the case of the bomb combustion, and the burning watched. The substances burned were filter paper (cellulose), cut in disks and laid on the bottom of the crucible, and powdered sugar (rock candy). After combustion the ordinary test was made for the oxygen by means of a glowing splinter of wood. In all cases there was a large excess of oxygen, since the large cork could be removed and the splinter plunged in and ignited several times in succession.

In the following table, which gives the results of the tests, crucible No. 1 was a large nickel crucible, $1\frac{5}{8}$ inches in diameter at the top and $1\frac{7}{16}$ inches deep. Crucible No. 2 was a small nickel crucible, $\frac{7}{8}$ inch in diameter by $\frac{11}{16}$-inch deep. Crucible No. 3 was the same as No. 2, perforated, and crucible No. 4 was the same as No. 3 with the holes enlarged. A small piece of cellulose, not weighed, was put under the sugar in the crucible in one case to facilitate the combustion. The time which the substance stood in the bottle before ignition was noted, as well as the length of time it continued to burn. The last column in the following table gives the unburned residue:

TABLE XIII.—*Powdered sugar and cellulose burned in different shaped crucibles in oxygen under atmospheric pressure.*

Substance.	Crucible.	Weight of substance.	Time before ignition.	Time of combustion.	Unburned residue.
		Gram.	*Minutes.*	*Seconds.*	*Gram.*
Cellulose	1	0.2190	30	60	0.0000
Sugar	1	.2675	5	75	.0077
Do	2	.2454	5	180	.0081
Cellulose	2	.1890	5	165	.0026
Sugar	3	.2582	8	195	.0122
Do	4	.2132	90	75	.1609
Sugar and cellulose	4	.2037	5	60	.0060
Cellulose	4	.1585	15	40	.0002

In only one instance did the substance burn without leaving any unburned residue of carbon. There was a marked difference between the two substances in the way they burned, the sugar taking longer time and leaving a great deal of unburned matter. A number of holes were made near the bottom of the small crucible. During the burning some of the holes became coated over with a film of charred material, the flame remained smaller, and the circulation of the gases, i. e., of oxygen, to the burning substance was lessened. When the holes were enlarged these last-mentioned hindrances were magnified and the sugar burned only in part, most of it was left untouched by the small flame, and in one spot it was not even fully melted. It is possible that in this case some of the oxygen might have leaked out on standing, but after the combustion there was enough left in the bottle to allow the glowing splinter to burst into flame several times. When the sugar rested on a small piece of filter paper the combustion of the sugar was more nearly complete, and filter paper alone was practically completely burned in crucible No. 4.

The figures of the table, as well as the actual behavior of the combustion, indicate the shape of the larger crucible, which was very much broader at the top than at the bottom, as being the best. The combustion itself, or we may say the supply of oxygen to the burning points, was more uniform with the larger and more intermittent with the smaller crucible. A good start, and much heat quickly generated, are factors which undoubtedly contribute much to make a combustion complete, but these may not in the least change the conclusion reached as to the shape of the crucible or capsule best suited for the work.

ALCOHOL HEAT VALUE.

Time did not permit me to take up the question of alcohol heat value as fully as I desired, hence at this time I shall make mention of only a few points which have come up in connection with the determination of heat of combustion, and make some suggestions which may at least be of practical value.

Alcohol being a volatile liquid it can not be satisfactorily burned in an open dish, but must be inclosed in a receptacle from which it can not evaporate, and gelatin capsules of known heat value are generally used for this purpose. The alcohol charge should never be weighed out by difference—i. e., by weighing the bottle—but the actual amount placed in the capsule should be weighed. The reason for this is to make sure that there is no evaporation. Unless the capsule with the alcohol is weighed a little evaporation may not be noticed, and if the capsule is not tight enough to hold the alcohol while it is being weighed it is not fit for the work. A little evaporation of alcohol from the capsule in the bomb will always cause the results to come too low, since alcohol vapors are not fully burned. To insure complete combustion of the alcohol and the gelatin the capsule may be filled with clean, ignited asbestos to absorb the alcohol.

Another way to obtain good combustions of the alcohol and gelatin, though not so simple as the gelatin-capsule method, is to make use of a tubular platinum capsule, about one-fourth of an inch in diameter, having perforated sides and bottom. This platinum capsule is coated with a mixture of the best gelatin with about 10 per cent of glycerin and water, all gently heated. The coating is allowed to dry, and the weight of the gelatin mixture is ascertained. Separate portions of the mixture are dried and analyzed for the heat value. This gelatin-coated capsule can be used with or without asbestos. Caps can be made of the same material to fit tightly over the end, or inverted, used as plugs. It requires less gelatin to coat such a capsule and make it alcohol tight than is found in the ordinary gelatin capsules used.

The weight of all gelatin capsules, etc., should be taken when the the material is dry, and this weight should be used in correcting for the heat of combustion of gelatin. But before using they should have been exposed to room conditions for a considerable time, else there is no constancy about them when they have to be reweighed and filled with alcohol.

ALCOHOL DETERMINATION USED FOR TESTING THE RESPIRATION CALORIMETER.

This was absolute alcohol diluted with distilled water, and hence the first step was to obtain its specific gravity. Two pycnometers were used and the specific gravity was taken at 15.6° C., as follows:

	No. 1.	No. 2.
	Grams.	*Grams.*
Pycnometer, plus distilled water	77.2663	87.2591
Pycnometer, empty	27.2385	37.2259
	50.0278	50.0332
50 cc. air	.05894	.05894
Water	50.08674	50.09214
Pycnometer, plus alcohol	68.4121	78.4056
Pycnometer, empty	27.2385	37.2259
	41.1736	41.1797
50 cc. air	.05894	.05894
Alcohol	41.23254	41.23864

No. 1. 41.23254÷50.08674=.823222 specific gravity.
No. 2. 41.23864÷50.09214=.823255 specific gravity.

Average, =.823238 specific gravity.
According to Squibb, at 15.6° C.
0.82755 specific gravity=88.0 per cent pure alcohol.
.81684 specific gravity=92.0 per cent pure alcohol.

Difference, .01071 specific gravity= 4.0 per cent pure alcohol.
0.82755 =88.0 per cent alcohol.
.823238= unknown alcohol.

.004312
0.01071 : 0.004312 :: 4.0 : X X=1.6105 per cent.
Hence 88.0+1.6105=89.611 per cent ethyl alcohol.

DETERMINATION OF HEAT OF COMBUSTION.

Using the old water value of the bomb and without any correction for oxygen, two determinations of the capsule gelatin gave an average of 4,871 calories per gram, and the average of three determinations of the alcohol gave 6,458.7 calories per gram, or 7,207 calories per gram pure alcohol. Applying the correction found for impurities in oxygen by burning 1 gram cellulose, and using the corresponding water value of the bomb, the average for the gelatin was 4,860 calories per gram, and for the alcohol 6,447 calories per gram, or 7,194 calories per gram pure alcohol.

A sudden jar of the bomb caused one capsule containing alcohol to fall to the bottom of the bomb. It was ignited, and the determination was carried through as usual, but only 6,949.8 calories per gram pure alcohol was measured—that is, 3.4 per cent less than the above average. Upon opening the bomb there was found a black spot and some yellowish oily liquid at the bottom, and a strong, very peculiar odor mixed with odor of alcohol was noticed, thus showing incomplete combustion very decidedly. Another charge of alcohol which had remained in the capsule in the bomb for several hours before ignition gave 7,091 calories per gram pure alcohol. Here, where there was opportunity for evaporation, the heat obtained was less than the average of the three other determinations.

These observations are sufficient to show that to obtain correct values great care must be taken when volatile substances are to be burned in the bomb.

One or two questions which may possibly prove to be of some importance in connection with this work will be considered briefly.

These questions concern the contents of the bomb before and after combustion, and their influence upon the measurement of the heat, namely, the influence which the changes in the contents have upon the water value of the bomb, and the quantity of heat held by these various compounds formed and not accounted for.

In the following calculations I shall use the values for specific heats, etc., as given below:

Per unit weight:
  Cane sugar=0.301 specific heat.
  Water =1.000 specific heat.
  O_2 = .2175 specific heat.
  CO_2 = .1875 specific heat.
  O_2 1 liter =1.4298 gram.

To illustrate these problems and for the sake of simplicity we shall consider one example, leaving out all of the minor things which would complicate the calculations. Thus, we have a bomb of 360 c. c. capacity. In the bomb, before combustion, are 1 gram cane sugar and 20 atmospheres pure oxygen, all at 20° C. What changes take place when the sugar is burned, and what are their significance?

According to the weight and specific heat of the substances in the bomb, they represent a certain mass of water at the same temperature; also, a definite quantity of heat is held by them, which may be expressed in calories. Twenty atmospheres oxygen would then be—

20×360 c. c.=7,200 c. c. oxygen.
7,200×1.4298 (weight of 1 liter oxygen)=10.29456 grams oxygen.
10.29456×0.2175 (specific heat)≎2.239 grams water.
1 gram cane sugar ×0.301 specific heat≎0.301 gram water.

Hence the substances in the bomb represent—

Oxygen	2.239 grams H_2O
Sugar	.301 grams H_2O
Total	2.540 grams H_2O

The sugar burns to CO_2 and H_2O, and after the combustion of 1 gram sugar we find 0.5793 gram water and 1.5426 gram CO_2.

1.5426 grams $CO_2 \times 0.1870$ specific heat$=0.288$ gram water.
$10.29456-1.1219=9.1727$ grams O_2, and $\times 0.2175=1.933$ grams water.

Thus in the bomb after combustion we have—

Oxygen equivalent to	1.933 grams H_2O
Carbon dioxide equivalent to	.288 gram H_2O
Water	.5793 gram H_2O
Total	2.8003 grams H_2O
After combustion	2.8003 grams water
Before combustion	2.5400 grams water
Difference (increase)	.2603 gram water

The whole bomb system therefore has changed to an extent equivalent to 0.2603 gram water during the combustion. This is equal to 0.011 per cent of the total bomb water value plus water used, or an error of about one-half calory in the ordinary determination. If, for instance, 1 gram butyl alcohol should be burned instead of sugar, the change in the system would be equal to 0.4685 gram water—an error of 0.019 per cent.

This is a very small error, and, at the present, is perhaps altogether negligible.

The next question is, To what extent does this change of contents affect the heat evolved during the combustion?

This question refers to the same changes in the contents of the bomb as did the preceding one, but instead of studying their effect upon the water value of the bomb we want to see what the effect will be upon the determination of heat of combustion when the factor for the bomb and the water used remains the same.

In the case of the sugar there was an increase of 0.2603 gram water in the bomb, which, raised to 1.7° C., would equal about one-half calory held by it. The correction will, in other words, in the case of sugar, be the same whichever way we apply it, but this is not necessarily true of all substances burned. As said before, the correction is so small that it need seldom be considered.

CONCLUSION.

From what has been said, then, we learn that there are many possibilities for error in the work with the bomb calorimeter. Undoubtedly many investigators in the past have worked with impure oxygen and never questioned its purity. In the light of our present experience it is questionable whether Stohmann himself, by the use of a heated copper tube, could have removed the last traces of combustible gases from his oxygen.

The disappearance of nitric acid formed and its relation to the ash has not been taken into consideration, and it is only within a couple of years that the thermometer lag has come to be applied in the calculations of the results.

These overlooked or at times unknown difficulties, which have been referred to throughout this paper, may be the cause of some of the disagreements of results as experienced by different investigators and referred to in the earlier part of this paper.

From what has been done and said I believe we also have learned that much work is yet needed in the different lines indicated before the method for determinations of heat of combustion by means of the bomb calorimeter can be called perfect.

Issued March 12, 1907.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN No. 95.
A. D. MELVIN, CHIEF OF BUREAU.

THE BACTERIOLYTIC POWER OF THE BLOOD SERUM OF HOGS.

BY

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1907.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., December 27, 1906.

Sir: I have the honor to transmit for publication the accompanying paper entitled "The Bacteriolytic Power of the Blood Serum of Hogs," by B. M. Bolton, M. D., of the Biochemic Division of this Bureau.

The experiments herein described were carried out with certain strains of the hog-cholera bacillus, and are a part of the general investigations concerning hog cholera which have been conducted for some years by the Biochemic Division. The subject of bacteriolysis has claimed much attention from bacteriologists in recent years, and Doctor Bolton's work is believed to be of interest on account of the light it throws upon the defensive mechanism of the animal body in its fight against infectious diseases.

I recommend that the paper be published in the bulletin series of this Bureau.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.

Introduction ... 7
  Definition of sera used in experiments ... 8
  Nature of the substance causing destruction of bacteria ... 9
Methods used in drawing blood, and description of cultures employed ... 14
The bactericidal potency of serum from the same hog at different times ... 15
Modifications which occur in the bactericidal power of serum upon standing ... 19
Comparison of the bactericidal potency of the same serum for different strains of bacteria ... 21
The potency of serum from arterial blood compared with that from venous blood ... 22
Effects of heat upon the bactericidal power of hog serum ... 24
Effects of injecting cultures of *B. choleræ suis* upon the bacteriolytic power of hog serum for this organism ... 27
Effects of dilution upon the bacteriolytic power of normal and immune serum ... 35
  Dilution of normal serum ... 35
  Dilution of immune serum ... 39
  Summary of the dilution experiments ... 45
General discussion ... 46
  Summary of the conditions affecting bacteriolysis ... 57
  The theory of complement diversion ... 58
Conclusions ... 60
Bibliography ... 61

ILLUSTRATIONS.

Page.

FIG. 1. Illustration of the mechanism of bacteriolysis according to the Ehrlich hypothesis 11

2. Diversion of complement in undiluted immune serum 13

3. Partial bacteriolysis and partial diversion of complement in diluted immune serum 13

4. Diversion of complement in immune serum, not heated, and without the addition of foreign complement 51

THE BACTERIOLYTIC POWER OF THE BLOOD SERUM OF HOGS.

INTRODUCTION.

Aside from a few preliminary experiments with cultures of the typhoid and colon bacilli, the present investigation was confined to the reactions taking place between three strains of *B. choleræ suis*, and normal and immune serum of hogs.

The bactericidal properties of the blood serum and other fluids of the body have of recent years attracted a great deal of attention from bacteriologists, and the investigations of the subject have led to very far-reaching results of both theoretical and practical importance. Without attempting to enumerate in full the different properties which blood serum has been found to possess in its various reactions with bacteria and their products, those properties which bear upon the present investigation may be briefly stated as follows:

The blood serum of many healthy animals when drawn into test-tubes has the power of destroying a larger or smaller number of bacteria when these are introduced into it. Thus large numbers of anthrax bacilli are destroyed by rabbit's blood serum in test-tube experiments. Typhoid, colon, and Asiatic-cholera bacteria are also destroyed by the blood serum of rabbits.

The same serum is not equally potent for different bacteria, and the serum from different animals of the same or of different species varies in bactericidal potency for the same organism. The chemical reaction of the serum seems to exert some effect upon this quality of the serum. The more alkaline the serum the more potent is its action apparently, and consequently the venous blood has been found sometimes to furnish more potent serum than the arterial blood from the same animal.[9] [a]

The injection of animals with the products of growth of bacteria produces different effects upon the properties of the blood serum, depending upon the kind of bacteria employed for injection. The injection of the bacteria of the group to which *B. choleræ suis*, *B. typhosus*, and *B. coli communis* belong appears sometimes to have no effect upon the bactericidal potency of the serum. At other times it seems to increase the potency, but frequently it has a very peculiar effect, for in some cases the serum after the injection of the animal will no longer kill any of the bacteria of the kind used to inject the animal, but on the dilution of the serum with an indifferent fluid, such as salt solution, it becomes strongly bactericidal for the bacteria of this kind. But this serum behaves like the serum from an uninoculated animal with other bacteria than those with which the animal is inoculated. Thus the serum from the blood of a rabbit injected with typhoid bacilli will often fail to kill the typhoid bacilli unless the serum is greatly diluted, but it will kill just as many Asiatic-cholera spirilla as it did before the animal was injected with the typhoid bacilli.[4] The peculiar behavior of the serum from an animal injected with a culture of bacteria, in its failure to kill the species of bacteria with which the animal is injected unless it is diluted, is called the Neisser-Wechsberg[14] pheuomenon, after the two investigators who first observed the reaction.

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[a] The figure references refer to bibliography at end of bulletin.

DEFINITION OF SERA USED IN EXPERIMENTS.

The serum obtained from an animal which has been injected with bacteria is spoken of as immune serum in contradistinction to the serum from an uninoculated animal, which is called normal or fresh serum. Thus serum obtained from a hog injected with *B. choleræ suis* would be known as *B. choleræ suis* immune hog serum. So wherever immune serum is mentioned in the present paper it is to be understood that it refers to the serum obtained from an animal which has received at least one injection of bacteria, but not necessarily that the serum has either immunizing or germicidal properties. On the other hand, where normal or fresh serum is mentioned, it is to be understood to refer to serum obtained from an uninoculated animal.

Serum loses in bactericidal potency on standing after being drawn from the animal; and the higher the temperature to which the serum is exposed the more rapid the loss of potency. It may remain potent for several days or for even a week or more in the refrigerator, but if kept at body temperature it generally loses all bactericidal properties in three or four hours. Heating at 55° or 56° C. for ten or fifteen minutes also robs the serum of its bactericidal power, or rather this treatment of immune serum suspends its bactericidal power, which is restored by the addition of a small amount of fresh serum. This suspension of bactericidal power by heating at 55° or 56° C. is called inactivating the serum, and wherever inactivated serum is mentioned it is to be understood to refer to serum which has been treated in this way. Inactivated serum to which fresh serum is added, and which has had its bacteriolytic properties restored in this way, is termed reactivated serum.

NATURE OF THE SUBSTANCE CAUSING DESTRUCTION OF BACTERIA.

In regard to the nature of the substance or substances in the serum which cause the destruction of the bacteria which are introduced, there is some difference of opinion. Some authorities maintain with Buchner that there is only one substance, called by him "alexin," which causes bacteriolysis, while others—and these appear to be in the majority—maintain that there are two substances concerned. Those who hold to the former view regard the effect produced by standing or by heating as due to the weakening of the alexin, while those holding the latter view explain this effect by a modification of one of the two substances which they regard as necessary for bacteriolysis. All are not agreed, however, as to the nature of these two substances. Bordet [1,2,3] and the French school generally look upon the substance which is not modified by heat as a sensitizing agent merely, which acts upon the bacteria in such a manner as to make them susceptible to the destructive action of the other body to which the bacteriolytic properties of the serum are directly due. Bordet borrows Buchner's term "alexin" for this active agent, and he gives to the other body the name "substance sensibilisatrice." According to the French school, then, bactericidal serum owes its power to two substances, called, respectively, substance sensibilisatrice and alexin. The former resists heating up to 75° C. or even somewhat higher temperatures for an hour or more, while the latter is destroyed by heating at 55° C. or higher in ten or fifteen minutes. Moreover, the substance sensibilisatrice according to this view is a specific substance in each case. The substance sensibilisatrice for one kind of bacterium sensitizes this one kind only, and while it can not of itself cause bacteriolysis, it nevertheless produces certain changes in the bacteria. On the other hand, according to the view of Ehrlich and his school, this body, the thermostabile body, causes no change in the bacteria themselves, but serves merely as an intermediary, a binding link, serving to connect the bacteria to the active body—the alexin of Bordet—which according to this view is the active agent in bacteriolysis. To the two hypothetical bodies concerned in the process Ehrlich has given, at different times, different names. At first he called the thermostabile body "intermediary body," afterwards "immune body," and finally "amboceptor." The last two designations are those now generally employed by the Ehrlich school for the body which corresponds, as is evident, to the substance sensibilisatrice of Bordet. To the other body, the alexin of the French school, Ehrlich has given variously the names "addiment" and "complement," the last being that now exclusively employed. The term intermediary body has been employed in a recent publication for the amboceptors present in the normal serum in order to distinguish these from immune amboceptors, or amboceptors produced by injecting animals with bacteria or their products.

But among those who have accepted more or less completely the Ehrlich conception of the nature of the bodies concerned in bacteriolysis there is difference of opinion as to the mode of action of the complement. Ehrlich himself says in regard to the matter "that one will not go amiss if he assumes with Pfeiffer that the process of bacteriolysis is analogous to digestion, and attributes to the addiment (complement) the character of a digestive ferment." Gruber,[8] on the contrary, contends that the complement does not act like a ferment, and that it is erroneous to draw any analogy between the complement and an enzym, since the complement is entirely used up in bacteriolysis, whereas in the process of fermentation, as is well known, the ferment is not used up, but may be recovered after the action is ended, and used for the fermentation of other material.

But whatever the exact mode of action may be, it is evident from what has just been said that both the Ehrlich and the Bordet schools attribute bacteriolytic action proper in normal serum to a substance easily changed by comparatively low temperatures, and called, respectively, complement and alexin by the two schools. To the other body concerned in bacteriolysis—the amboceptor of Ehrlich and the substance sensibilisatrice of Bordet—is assigned by the former the rôle of a binding link between the complement and the bacterium, while by the latter is assigned to it the property of a sensitizer, or of a mordant as in dyeing. In the one case the bacterial cell is regarded as not at all injured or otherwise changed by the union with the amboceptor; in the other case it is the opinion of those holding this view that the cell is acted upon and changed by the sensibilisatrice in such a way that the alexin can penetrate it.

Bordet[2] summarizes the difference between his theory and that of Ehrlich as follows:

> According to Ehrlich and Morgenroth the specific antibody (sensibilisatrice) plays the rôle of an actual intermediary (zwischenkörper, amboceptor), a link of union attaching itself on the one hand to the cell, on the other to the alexin. In other words, the absorption which the alexin undergoes in the presence of the sensitized cell is not due to an affinity manifested by the cell itself to this substance. The absorption of the alexin is only indirect; the cell joins itself to the intermediary substance, which is itself united chemically by its other pole to the alexin.
>
> Our idea of the phenomenon, which we feel we are justified in holding, is altogether different from this. To us it seems that the sensibilisatrice which unites with the cell modifies this in a way which permits it to absorb the alexin directly. The action of the sensibilisatrice upon the cells is comparable to that of certain fixative agents or mordants which confer upon certain substances (or to the cells of these substances, as is the case in histological technic) the power of absorbing colors which they refuse to absorb before treatment. * * * It is to be clearly understood, however, that when we speak of mordants in this connection we do not intend to apply in all details the phenomena of dyeing to the matter at present under consideration; we merely mean to draw a comparison which will serve to make our

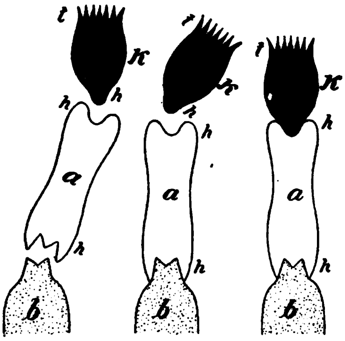
> idea clearer. The hypothesis which we wish to bring out in relief is that in the presence of hemolytic serum, the cell becomes capable of absorbing directly the alexin by means of its own proper elective affinity, and that this power is due to a change caused by the sensibilisatrice. In other words, we do not believe that one is forced to admit, with Ehrlich and Morgenroth, that the sensibilisatrice itself combines with the alexin, and that this union is indispensable for the latter substance to attack the cell.

Bordet furthermore states in the same connection that he agrees with Buchner in regarding the alexin for blood cells and for bacteria as identical—that one and the same alexin may attack the most diverse cells; whereas Neisser and others of the Ehrlich school believe that alexins or complements are different in one and the same serum.

While it is evident from the above that the terms amboceptor and sensibilisatrice are used to designate the same substance, it is scarcely correct to use them interchangeably, since they connote somewhat different attributes in the body to which they refer. The same is true of the terms complement and alexin, though to a less degree.

The following diagrams, obtained from various sources and modified to suit the purpose, will serve to illustrate the process of bacteriolysis according to the views of the Ehrlich school.

Figure 1 represents in its simplest form the mechanism of bacteriolysis according to the Ehrlich hypothesis, and serves to illustrate the process sufficiently for the purposes of the present paper.



1 2 3

FIG. 1.—Illustration of the mechanism of bacteriolysis according to the Ehrlich hypothesis.

In the diagram the bacteria are represented by the parts marked *b*, the amboceptors by those marked *a*, and the complements by those marked *k*. In No. 1 the bacterium, amboceptor, and complement are represented as just on the point of uniting. No. 2 represents the bacterium and the amboceptor united and the complement on the point of uniting with the unoccupied end of the amboceptor. No. 3 represents the process of uniting of bacterium, amboceptor, and complement completed; the bacterium in this case would undergo bacteriolysis.

It should be borne in mind that according to this theory bacteriolysis can take place only where the bacterium becomes united to an amboceptor which is itself united with a complement. A bacterium may become united with a free amboceptor—i. e., an amboceptor which is not united with a complement—but the bacterium in such a case does not undergo bacteriolysis unless a complement subsequently becomes attached to the amboceptor. The complement is incapable of uniting directly with a bacterium; it can do this only through the intervention of the amboceptor. But when the complement becomes linked to the bacterium by means of the amboceptor the bacterium becomes broken up into minute granules and ultimately disappears.

The bonds by which the amboceptor attaches itself to the bacterium on the one hand, and to the complement on the other, are called haptophor groups or haptophors (*h*), and similarly this name is given to the bonds of union of the bacteria and of the complement. The amboceptor thus has two haptophors, one by means of which it attaches itself to the bacterium, the cytophylic haptophor, and one by means of which it attaches itself to the complement, the complementophylic haptophor. The bacteria probably possess each many haptophors all of the same kind—i. e., haptophors capable of uniting with amboceptors of the same kind—but for the sake of simplicity the bacterium is represented in the diagram as having only one haptophor. The complement has one haptophor group and one so-called toxophor group (*t*), and it is by means of the latter group that the complement acts upon the bacterium. The complement may be deprived of this toxophor group, and although it is still capable of uniting with the amboceptor in such a case, it can no longer cause bacteriolysis. This loss of the toxophor group is caused by heating, and it also occurs spontaneously in the serum on standing. Bacteria subjected to the action of heated serum do not undergo bacteriolysis, but become fixed to the amboceptors, and the amboceptors become united to the haptophor group of the complement which are left unaffected by the heating. It will thus be readily understood why bacteria treated with heated immune serum are subsequently protected from bacteriolysis even when unheated immune serum or when unheated complement is added to them. The complementophylic haptophor of the amboceptor is in such a case already occupied by the haptophors of the heated complement, which has in this way become deprived of its toxophor group.

The amboceptors found in ordinary normal serum are either all alike—and in this case they must possess affinity for a great many different kinds of bacteria—or they must differ from one another; and in this case there must evidently be a great many specific amboceptors, some fitted for the bacteriolysis of one species of bacterium, some for others. This matter seems not yet to have been settled. But it is certain that the injection of an animal with certain bacteria or their products causes the formation of a large number of specific amboceptors; that is to say, of amboceptors having affinity only for the kind of bacteria with which the animal is injected. Such injections seem not to increase the amount of complement.

Complement is found normally in the serum, that of some animals possessing more than that of others. The horse appears to have a large amount of complement in the serum. It is not yet settled whether the complement is specific—that is, whether the complement for one kind of immune serum can unite with the amboceptors of this serum only and not with the immune serum of a different sort—or whether complements are general; though they seem for the most part not to be specific. The complement in the serum of horse's blood seems capable of reactivating heated immune serum of various kinds. Still in some cases it would appear as if they were specific.

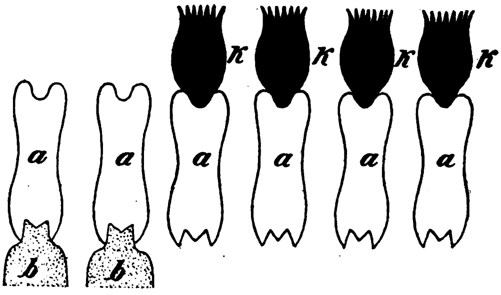


FIG. 2.—Diversion of complement in undiluted immune serum.

With the explanation given above of the nature of amboceptors and complements, the phenomena which take place in immune serum become more or less satisfactorily explicable. By means of the characteristics ascribed to these bodies it is possible to account for the peculiar behavior of immune serum stated above, consisting in the fact that such serum is frequently more potent when diluted than when it is undiluted.

Neisser and Wechsberg were the first to observe this phenomenon, and the theory which they advance to explain it they very appropriately call the theory of the diversion of complement. As the name implies, they attribute the lack of bacteriolysis in the undiluted immune serum to the turning aside of the complement from the bacteria, or rather from the amboceptors which are attached to the bacteria. They hold that this diversion is brought about by the free amboceptors themselves. In other words, where there are more amboceptors than there are complements present in a serum, a part of these attach themselves to the bacteria and a part to the complements.

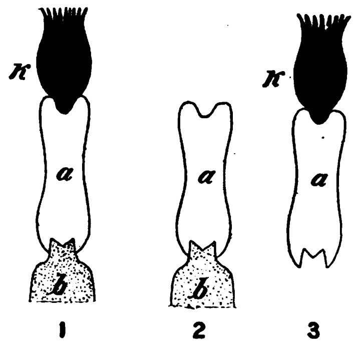


FIG. 3.—Partial bacteriolysis and partial diversion of complement in diluted immune serum

The accompanying diagram (fig. 2), taken from Neisser and Wechsberg, and modified to suit the present description, shows two amboceptors *a*, attached to bacteria *b*, and four amboceptors attached to complements *k*. Bacteriolysis is not possible in such a condition, because the complements have been diverted from the amboceptors which are attached to the bacteria. Bacteriolysis can take place only when the complement becomes attached to the bacterium through the medium of the amboceptor.

Figure 3 is meant to show the same serum diluted with an equal amount of salt solution. In this case, with the same number of bacteria added, it is evident that one-half of them would be killed, as is indicated by the combination between bacteria, amboceptor, and complement in No. 1. The other half of the bacteria would evidently escape, the complement being diverted by the free amboceptor, as shown by Nos. 2 and 3.

Although these statements in regard to bacteriolysis and the mechanism probably involved are by no means exhaustive, they will, perhaps, serve the purposes of the present investigation and to explain the results here obtained. It will be noticed that experiments have been made with a view to throwing additional light upon those phases of bacteriolysis already mentioned, as they were observed in hog's blood serum, and it would appear that the results, although differing in some instances from those of other observers, are nevertheless susceptible of interpretation in harmony with more or less firmly established hypotheses.

METHODS USED IN DRAWING BLOOD, AND DESCRIPTION OF CULTURES EMPLOYED.

The blood was drawn from healthy hogs, or from hogs injected with cultures of *B. choleræ suis*, subcutaneously or intravenously, as stated in each case, at various lengths of time before the drawing. It was obtained (1) by cutting off a piece of the tail and allowing the blood to flow into sterilized tubes or flasks, (2) by bleeding from an artery in the ear, or (3) by inserting a canula into the carotid artery or jugular vein. In some cases the blood was drawn from the carotid and from the jugular at the same operation. After drawing, the blood was usually placed in the refrigerator to allow the serum to separate, but in a few cases it was used immediately after drawing.

All the hogs used in the experiments were in a perfectly healthy condition to start with, and, as far as could be ascertained, had never been sick. They weighed from 30 to 40 pounds each.

The cultures employed were obtained during the course of former experiments. One of them, G. P. 4692, had been repeatedly passed through guinea pigs; another, Crawford, had been carried along for a number of years on artificial media without passage through animals; the third, F. 26, had also been carried along for several years on artificial media without animal passage. These cultures presented minor points of difference from one another, but they were all quite typical for *B. choleræ suis*. All of them give characteristic growths upon artificial culture media with the usual fermentation reactions of the sugars, and the other features of *B. choleræ suis*, both macroscopic and microscopic, though it is true that the Crawford strain grew more vigorously and gave larger and denser colonies than the other two, and that the G. P. 4692 strain gave the least vigorous growth on artificial media. Also, in the tests which were made to determine the point, the G. P. 4692 strain was much more strongly pathogenic for guinea pigs, rabbits, and hogs than the other two strains. The Crawford strain was the least virulent of the three. So the virulence of the three organisms was in inverse ratio to the vigor of growth upon artificial culture media. The culture of *B. coli communis* was obtained in the course of former experiments from the feces of a normal hog. It showed no pathogenic properties.

THE BACTERICIDAL POTENCY OF SERUM FROM THE SAME HOG AT DIFFERENT TIMES.

The bactericidal potency of the serum from the same hog on different days was tested by bleeding a hog at intervals of a few days, drawing off the serum after the blood had stood in the refrigerator for twenty-four hours or less, and distributing it into test tubes—1 c. c. into each tube—then adding a definite, measured amount of a suspension in salt solution of the bacteria to be tested. At the same time a tube of 1 c. c. of salt solution was inoculated with the same measured amount of bacterial suspension and plates made immediately to determine the number of bacteria added. Plates were made from the serum tubes on the following day. The serum from 4 hogs was used in this experiment, the blood in all four experiments being drawn from the tail. The results will be found in the following tables:

Table I.—*Bacteriolytic action of normal hog serum from the same animal at different drawings. Blood drawn from the tail. Hog No. 1733.*

Date.	Time after drawing.	No. of drawing of blood.[a]	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.					
April 7	3 hours	I	G. P. 4692	4,620	79,800
7	.....do	I	*B. coli*	5,880	30,800
9	.....do	II	G. P. 4692	30,800	48,300
9	.....do	II	*B. coli*	7,000	0
11	1 day	III	G. P. 4692	4,160	9,800
11	.....do	III	*B. coli*	225	53
14	.....do	IV	G. P. 4692	9,800	4,690
14	.....do	IV	*B. coli*	9,520	9,800
19	.....do	V	G. P. 4692	8,655	142,900
19	.....do	V	*B. coli*	2,600	0
22	2 days	VI	G. P. 4692	4,968	43,200
22	.....do	VI	*B. coli*	1,940	240
26	1 day	VII	G. P. 4692	1,800	1,220
26	.....do	VII	*B. coli*	2,800	16,870
May 3	3 hours	VIII	G. P. 4692	22,628	17,510
3	.....do	VIII	*B. coli*	23,380	84
5	.....do	IX	G. P. 4692	8	1,004
5	.....do	IX	*B. coli*	869	2,594

[a] Roman numerals denote the serial numbers of the drawings of blood.

As will be seen from Table I, the blood was drawn from hog 1733 on nine different days and tested simultaneously upon cultures of G. P. 4692 and on the culture of *B. coli communis*. In four of the drawings the serum apparently had no bacteriolytic power for the strain of *B. choleræ suis* employed, while in four of them, although there was also no marked bacteriolysis, there was nevertheless no evidence of any great increase of the bacteria introduced. In the serum from one of the drawings the bacteria were reduced to one-half of the number introduced; this was the greatest amount of potency shown in any of the drawings.

With the colon bacillus there was marked bacteriolysis in one drawing, less marked in four other drawings, and neither increase nor decrease in one of the drawings, while in the other three drawings there was an increase of the bacteria introduced.

It is evident that the serum from this hog showed different degrees of potency or absence of potency upon the different occasions when the blood was drawn.

Table II.—*Bacteriolytic action of normal hog serum from the same animal at different drawings. Blood drawn from the tail. Hog No. 1740.*

Date.	Time after drawing.	No. of drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.					
May 6	3 hours	I	G. P. 4692	4	1,750
6	.....do	I	*B. coli*	975	8
8	.....do	II	G. P. 4692	4,925	1,276
8	.....do	II	*B. coli*	3,778	70
9	.....do	III	G. P. 4692	102	1,236
10	.....do	IV	G. P. 4692	1,010	1,552
10	.....do	IV	*B. coli*	3,110	994
11	.....do	V	G. P. 4692	848	450
12	.....do	VI	G. P. 4692	138	0
12	.....do	VI	*B. coli*	1,700	1,840

The blood of hog 1740, as is shown in Table II, was drawn on six different days and tested on *B. coli* and on G. P. 4692. It showed marked bactericidal power for the *B. coli* culture in three of the four drawings tested with this organism, but in one there was no decrease in the number of bacteria introduced. With the *B. choleræ suis* culture it will be noticed from the table that the effect varied greatly with the different drawings. While there was no very marked decrease in any case, it is evident in general that the bacteria did not multiply abundantly in the serum in any case. It would seem as if growth had been inhibited by the serum, although active bacteriolysis was lacking except in the serum from one drawing.

Table III.—*Bacteriolytic action of normal hog serum from the same animal at different drawings. Blood drawn from the tail. Hog No. 1741.*

Date.	Time after drawing.	No. of drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.					
May 8	3 hours	I	G. P. 4692	4,925	82
8	.....do	I	*B. coli*	3,778	14
9	.....do	II	G. P. 4692	102	166
9	.....do	II	*B. coli*	1,970	390
10	.....do	III	G. P. 4692	1,010	1,932
10	.....do	III	*B. coli*	3,110	40
11	.....do	IV	G. P. 4692	848	50
12	.....do	V	*B. coli*	1,700	0

As Table III shows, the blood was drawn from hog 1741 on five different occasions. The serum from four of the drawings was tested upon the culture of *B. coli*, and it showed marked bacteriolytic properties for this organism in all the tests. The serum from four of the drawings was tested upon the G. P. 4692 strain of *B. choleræ suis*, but only two of the drawings showed bacteriolytic properties for this organism.

TABLE IV.—*Bacteriolytic action of normal hog serum from the same animal at different drawings. Blood drawn from the tail. Hog No. 1742.*

Date.	Time after drawing.	No. of drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c.c.	Number of bacteria per 1 c c. after 1 day.
1905.					
May 8	3 hours	I	G. P. 4692	4,925	82
8	....do	I	*B. coli*	3,788	686
10	....do	II	G. P. 4692	102	46
10	....do	II	*B. coli*	1,970	82
12	....do	III	G. P. 4692	1,010	798
12	....do	III	*B. coli*	3,110	30
11	....do	IV	G. P. 4692	848	50
13	....do	V	*B. coli*	1,700	0
23	....do	VI	G. P. 4692	10,880	3,376
23	....do	VI	Crawford	40,500	2,640
23	....do	VI	F. 26	19,040	18,280
24	....do	VII	G. P. 4692	1,682	0
24	....do	VII	Crawford	1,808	180
24	....do	VII	F. 26	12,040	1,640
26	....do	VIII	Crawford	320	0
26	....do	VIII	F. 26	80	1,960
29	....do	IX	F. 26	1,740	4,480
29	....do	IX	Crawford	2,320	0
29	....do	IX	G. P. 4692	1,920	960
June 5	½ hour	X	F. 26	2,840	26,640
5	....do	X	Crawford	3,380	180
5	....do	X	G. P. 4692	1,380	4,420

In the experiments with the blood of hog 1742, as shown in Table IV, ten different drawings were made on separate days, and four of them tested on the culture of the colon bacillus, eight on culture G. P. 4692, five on culture Crawford, and five on culture F. 26.

The colon bacillus was greatly diminished in all cases. In one case where 1,700 per 1 c. c. had been introduced into the serum all of them were destroyed, and in another test 3,000 out of 4,000, in round numbers, were killed.

The tests with the G. P. 4692 culture showed that the serum was bactericidal in seven tests out of eight. In one of these about 1,700 bacilli were introduced and all of them were killed. On the other hand, in the serum from one of the drawings there was an increase of the bacilli introduced.

The tests with the Crawford culture showed that the serum from all of the drawings tested was actively bactericidal, but in some more than in others. In one of the drawings about 38,000 bacilli per 1 c. c. of serum were destroyed out of the 40,000 introduced. But in another case all were not destroyed although only 1,808 were introduced.

Tests with culture F. 26 showed bacteriolysis in one drawing; in another drawing there was no increase nor decrease of the bacteria introduced. In three drawings there was marked increase of the organisms introduced.

That the bactericidal potency of the serum of hogs should vary at different times, as these results seem to indicate, should perhaps not be a matter of surprise. There are probably many circumstances which influence this property of the serum. As is stated elsewhere in this paper, the chemical reaction of the serum has been found by others to influence the bactericidal power, and doubtless there are other as yet obscure circumstances which raise or lower this power of the serum. The nature of bacteriolysins will be found discussed at some length below in a different connection, and it would not seem at all improbable that there may be more of these at one time than at another present in the blood serum. Indeed, the production of bacteriolysis in serum of the living animal seems to be easily influenced one way or another, and it would not be unreasonable to regard them as varying from time to time under even slightly changing conditions of the body.

Trommsdorf[19] noticed that human sera derived from normal individuals as well as from those suffering from various diseases vary greatly in bacteriolytic power. Petterson[15] found the same thing with chickens.

Morgenroth and Sachs[13] found great variation in cytolytic power in serum of various sorts. Thus the serum from a horse at one drawing was hemolytic for rabbits' corpuscles but not for those of guinea pigs; three days later the serum from the same horse was strongly hemolytic for guinea pigs' corpuscles, but only very slightly for rabbits' corpuscles; twenty-three days later the serum from this horse was not hemolytic for guinea pigs' corpuscles, but strongly hemolytic for rabbits' corpuscles.

It is therefore evident that the cytolytic power of serum is very variable. Not only does the blood from different individuals of the same species differ in this respect, but the serum from the same individual differs from time to time. This is probably the case with all animals, and, as is apparent in the experiments with hogs, these are no exception to the rule.

In regard to the above experiments, while there is more or less variation between single tests, if one strain only of *B. choleræ suis* is considered, there seems to be a general tendency toward either active bacteriolysis or else inhibition of growth.

MODIFICATIONS WHICH OCCUR IN THE BACTERICIDAL POWER OF SERUM UPON STANDING.

As has been previously stated, the blood was placed on ice in all cases where it was not at once used for making tests. In some cases the bactericidal properties of the serum were tested after the serum had stood for various lengths of time. The results of these tests, as will be seen from the accompanying tables, show in general that the bactericidal potency is retained in some cases for as long as nine days. Occasionally, however, the bactericidal power of the serum is diminished by even two days' standing.

Table V.—*Bacteriolytic action of normal hog serum after standing for various lengths of time in the refrigerator. Blood drawn from the tail, eighth drawing. Hog No. 1742.*

Date.	Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.				
May 26	3 hours	Crawford	320	0
June 2	7 days	do	1,960	380
May 26	3 hours	F. 26	80	1,960
June 2	7 days	do	3,280	2,980

The serum from the blood of hog 1742 at the eighth drawing, as is shown in Table V, was tested upon two of the organisms only, namely, Crawford and F. 26. For the Crawford culture the bactericidal power of the serum was retained for seven days, whereas for the F. 26 culture the serum was bactericidal at the start and only inhibitory after seven days.

Table VI.—*Bacteriolytic action of normal hog serum after standing for various lengths of time in the refrigerator. Blood drawn from the tail, ninth drawing. Hog No. 1742.*

Date.	Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.				
May 29	3 hours	F. 26	1,740	4,480
June 3	5 days	do	1,220	1,180
May 29	3 hours	Crawford	2,320	0
June 3	5 days	do	1,060	0
May 29	3 hours	G. P. 4692	1,920	960
June 3	5 days	do	700	1,100

At the ninth drawing from hog 1742, as Table VI shows, the serum retained its bactericidal potency for the Crawford culture apparently unabated for five days, whereas for the other two organisms there was apparently more or less inhibition of growth, perhaps, both at the start and after five days, but no very marked bacteriolysis for either of them.

TABLE VII.—*Bacteriolytic action of normal hog serum after standing for various lengths of time in the refrigerator. Blood drawn from the tail, tenth drawing. Hog No. 1742.*

Date.	Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c.c. after 1 day.
1905.				
June 5	½ hour	F. 26	2,840	26,640
6	1 day	.....do	2,240	51,760
7	2 days	.....do	3,440	900
8	3 days	.....do	2,200	1,340
9	4 days	.....do	1,360	18,280
19	14 days	.....do	4,040	[a] ∞
5	½ hour	Crawford	3,380	180
6	1 day	.....do	5,200	120
7	2 days	.....do	7,440	760
8	3 days	.....do	3,500	3,160
9	4 days	.....do	2,640	1,380
19	14 days	.....do	1,440	∞
5	½ hour	G. P. 4692	1,380	4,420
6	1 day	.....do	3,040	6,320
7	2 days	.....do	180	3,420
8	3 days	.....do	360	2,000
9	4 days	.....do	1,880	∞
19	14 days	.....do	1,740	∞

[a] This character signifies that there were too many colonies to count.

The serum from the blood at the tenth drawing from hog 1742, as Table VII shows, preserved its bactericidal power to a marked degree for the Crawford culture up to and including the second day of standing. It appears to have been somewhat more potent on the fourth than on the third day of standing, but the difference in potency of the serum on these two days is hardly sufficient, perhaps, to be of any great significance. For the other two strains there was at most inhibition shown in some of the tests, but no decided bacteriolysis either before or after standing, except perhaps in the case of the F. 26 strain after two days. But in the case of this organism the serum appeared to have been more potent after standing for three days than it was previous to this time.

TABLE VIII.—*Bacteriolytic action of normal hog serum after standing for various lengths of time in the refrigerator. Blood drawn from the tail, eleventh drawing. Hog No. 1742.*

Date.	Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
1905.				
June 12	½ hour	F. 26	2,580	650
16	4 days	.....do	1,000	1,180
19	7 days	.....do	4,040	16,500
22	10 days	.....do	26,000	[a] ∞
12	½ hour	Crawford	2,560	400
16	4 days	.....do	2,860	280
19	7 days	.....do	1,440	320
22	10 days	.....do	50,400	∞
12	½ hour	G. P. 4692	15,120	800
19	7 days	.....do	1,740	1,280
22	10 days	.....do	8,880	∞

[a] This character signifies that there were too many colonies to count.

The serum from the blood of hog 1742 at the eleventh drawing, as shown in Table VIII, had marked bactericidal power for all three strains at the start, and this was retained for as long as seven days for the Crawford culture, whereas there was inhibition only for F. 26 after four days, and after this there was not even inhibition for this organism. The effect upon the G. P. 4692 culture was similar to that upon the F. 26 strain.

TABLE IX.—*Bacteriolytic action of normal hog serum after standing for various lengths of time in the refrigerator. Blood drawn from the jugular vein, twelfth drawing. Hog No. 1742.*

Date.	Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c.c.	Number of bacteria per 1 c.c. after 1 day.
1905.				
June 22	½ hour	Crawford	4,320	[a] ∞
23	1 day	do	4,060	∞
22	½ hour	F. 26	3,380	∞
23	1 day	do	640	∞
22	½ hour	G. P. 4692	1,240	∞
23	1 day	do	340	∞

[a] This character signifies that there were too many colonies to count.

The serum from the blood of hog 1742 at the twelfth drawing (Table IX) was taken from the jugular vein, and it will be observed from the table that the serum showed no bacteriolytic power either at the start or on standing. In order to test whether the lack of bactericidal power was due in this case to the fact that the blood was drawn from the vein, experiments, which are hereinafter given (page 22), were made to compare the bactericidal power of arterial blood serum with that of venous blood serum.

COMPARISON OF THE BACTERICIDAL POTENCY OF THE SAME SERUM FOR DIFFERENT STRAINS OF BACTERIA.

If in the foregoing tables comparison is made between the results obtained with the same drawing of blood upon the various organisms, it will be seen that quite decided differences appear.

Selecting the most striking contrasts, in one case there was a decided increase of the *B. choleræ suis*, culture G. P. 4692, while in the same blood 7,000 *B. coli* per 1 c. c. were all destroyed. It is true that in this case a great many more of *B. choleræ suis* were introduced at the start than of *B. coli*, and this may have influenced the result somewhat. But in another case where approximately the same number of organisms of the two kinds were introduced at the start there were very few of the *B. choleræ suis*, if any, destroyed, whereas nearly all of the *B. coli* were destroyed. In still another case there was a very great increase of *B. choleræ suis*, while all of the *B. coli* were destroyed. In one case there was no notable increase of the *B. choleræ suis*, while there was a decrease of the *B. coli.*

It is evident, therefore, that the bactericidal power of hog serum is different for this strain of *B. choleræ suis* and for the *B. coli*, in so far as this can be determined by the methods at present in use.

THE POTENCY OF SERUM FROM ARTERIAL BLOOD COMPARED WITH THAT FROM VENOUS BLOOD.

As hereinbefore stated, the following experiments were undertaken because of the results obtained with the serum from the venous blood in the experiment in which it appeared that there was neither inhibitory nor bactericidal power in the serum. In the experiments previous to this one the blood was drawn from the tail, and was consequently mainly arterial. It spurted from a severed artery when the tail was cut. In the experiments at present under consideration the blood was drawn from the carotid artery or from the jugular vein either at the same operation or on different occasions.

TABLE X.—*Bacteriolytic action of arterial serum compared with that of venous serum, both sera from the same normal hog (No. 1733) at the same operation. Tenth drawing of blood. Tests made at intervals after drawing.*

Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.	
			Arterial serum.	Venous serum.
1 day	F. 26	3,880	60	320
2 days	do	4,920	16,320	600
9 days	do	380	2,200	400
1 day	Crawford	940	40	80
2 days	do	1,480	200	120
9 days	do	420	140	40
1 day	G. P. 4692	2,440	9,760	5,920
2 days	do	9,880	8,160	6,560
9 days	do	300	100	140

The serum from hog 1733 at the tenth drawing, as shown in Table X, was taken at the same operation from the carotid artery and from the jugular vein. In the tests with the Crawford culture the serum from the arterial, as well as that from the venous blood, seem both to have possessed considerable potency, and to have possessed it in about equal degree. They furthermore appear to have retained their potency, much or all of it, for nine days. For G. P. 4692 culture neither serum appears to have had strong bactericidal power in any case. They both seem to have had some inhibitory power for this organism both at the start and after nine days. For culture F. 26 there appears to have been no difference between the two kinds of serum after having stood for one day, but the serum from the venous blood would seem to have retained its potency upon standing more tenaciously than that from the arterial blood.

TABLE XI.—*Bacteriolytic action of arterial serum compared with that of venous serum, both sera from the same normal hog (No. 1733) at the same operation. Eleventh drawing of blood. Tests made at intervals after drawing.*

Time after drawing.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.	
			Arterial serum.	Venous serum.
1 day	F. 26	5,420	10,480	220
2 days	do	6,520	20,160	27,680
5 days	do	8,580	280	8,960
8 days	do	240	10,960	2,600
1 day	Crawford	8,160	160	10,860
2 days	do	8,660	400	360
5 days	do	26,240	20,640	580
8 days	do	1,920	7,280	60
1 day	G. P. 4692	2,160	2,520	8,300
2 days	do	2,720	2,960	8,340
5 days	do	[a] ∞	21,240	1,360
8 days	do	2,240	∞	2,820

[a] This character signifies that there were too many colonies to count.

The blood obtained at the eleventh drawing from hog 1733, as shown in Table XI, was drawn from the artery and from the vein at the same operation. There seems to have been a difference in the potency between the two kinds of serum for all three organisms. For the Crawford culture the serum from the arterial blood seems to have been quite strongly bactericidal at the start, but the serum from the venous blood was apparently not so. The tests made after the serum had stood for two days would indicate that both kinds of serum were potent. The serum from the venous blood in this case seems to have gained in potency upon standing, a result previously noted in the case of culture F. 26 with a different serum, but it seems necessary to conclude that an error of some kind must have crept in where such results are obtained, since they are at variance not only with the a priori probabilities of the case but also with the usual experience. On the whole, the results in this experiment seem to indicate for the Crawford culture a greater potency, or at least a retention of a greater amount of potency, on the part of the venous than of the arterial blood.

For culture F. 26 in this experiment the arterial serum appeared less potent at the start and after eight days than the venous serum, but after standing five days it was more potent than the venous serum. Here again the serum seems to have gained potency on standing.

For G. P. 4692 the stronger retention of potency of the venous serum as compared with the arterial is apparent in the tests made five days and eight days after drawing. No difference between the two sorts of serum is noticeable in the tests made with this organism one day and two days after drawing.

The fact that blood through which a stream of CO_2 was conducted becomes more alkaline led Hamburger[9] to make tests of the comparative bactericidal potency of serum from ordinary blood and that from blood through which CO_2 had been passed, as well as of the difference in this respect between arterial and venous blood. He found, in fact, that serum from venous blood and from blood through which CO_2 had been passed is more potent than arterial-blood serum.

EFFECTS OF HEAT UPON THE BACTERICIDAL POWER OF HOG SERUM.

A large number of tests were made parallel with those above recorded to determine the effect of heat upon the germicidal power of hog serum, and also to determine whether there is any difference between the potency of the heated serum for the different strains of *B. choleræ suis* upon which it was tested.

The method employed in making the tests consisted in immersing the tubes containing the serum up to the plugs in a water bath which was kept by means of a thermoregulator exactly at the temperature to be tested. The results are shown in the following tables, from which it will be seen that the serum was subjected to temperatures of 51°, 52°, 53°, and 54° C. for ten minutes and for thirty minutes at different times. Tests were also made at temperatures above 54° C., but these are not tabulated for the reason that they gave results similar to those at 54°.

TABLE XII.—*Effects of heat upon the bacteriolytic power of normal hog serum. Blood drawn from the tail and kept in the refrigerator for various lengths of time. Temperature employed, 51° C.*

Date.	Time after drawing.	No. of hog and drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Arterial blood. Number of bacteria per 1 c. c. after 1 day. Not heated.	Arterial blood. Number of bacteria per 1 c. c. after 1 day. Heated.	Length of exposure to heat.
1905.							
April 7	3 hours	1733 I	G. P. 4692	4,620	70,800	49,000	10 minutes.
	do	do	*B. coli*	5,880	30,800	36,400	Do.
June 12	½ hour	1742 XI	F. 26	2,850	650	96,600	30 minutes.
	4 days	do	do	1,000	1,180	1,160	Do.
	7 days	do	do	4,040	16,500	[a] ∞	Do.
	10 days	do	do	26,000	∞	∞	Do.
	½ hour	do	Crawford	2,560	400	440	Do.
	4 days	do	do	2,860	280	4,440	Do.
	7 days	do	do	1,440	320	4,200	Do.
	10 days	do	do	50,400	∞	∞	Do.
	½ hour	do	G. P. 4692	15,120	800	11,200	Do.
	7 days	do	do	1,740	1,280	1,840	Do.
	10 days	do	do	8,880	∞	28,560	Do.

[a] This character signifies that there were too many colonies to count.

It would appear from the results recorded in Table XII that heating for ten minutes at 51° C. produced no effect in the two tests which were made in this manner, one on the G. P. 4692 strain of *B. choleræ suis*, and the other on the colon culture. The same temperature for thirty minutes robbed the serum of much or all of its power in 8 of the 11 tests.

TABLE XIII.—*Effects of heat upon the bacteriolytic power of normal hog serum. Blood drawn from the tail. Temperature employed, 52° C.*

Date.	Time after drawing.	No. of hog and drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.				Length of exposure to heat.
					Arterial blood.		Venous blood.		
					Not heated.	Heated.	Not heated.	Heated.	
1905.									
June 28	1 day	1733 X	F. 26	3,880	60	1,540	320	10,720	30 minutes.
	2 days	do	do	4,920	16,320	15,200	600	3,360	Do.
	9 days	do	do	380	2,200	1,720	400	[a] ∞	Do.
	1 day	do	Crawford	940	40	3,960	80	25,080	Do.
	2 days	do	do	1,480	200	460	120	∞	Do.
	9 days	do	do	420	140	300	40	∞	Do.
	1 day	do	G. P. 4692	2,440	9,760	1,920	5,920	3,080	Do.
	2 days	do	do	9,880	8,160	9,840	6,560	10,000	Do.
	9 days	do	do	300	100	100	140	....	
July 12	1 day	1733 XI	F. 26	5,420	10,480	....	220	5,680	Do.
	2 days	do	do	6,520	20,160	14,880	27,680	41,720	Do.
	1 day	do	G. P. 4692	2,160	2,520	2,440	3,300	2,240	Do.
	2 days	do	do	2,720	2,960	2,180	3,340	(?)	

[a] This character signifies that there were too many colonies to count.

It will be seen from the above table that the effect of an exposure of thirty minutes showed a marked influence in some cases while in others it was apparently without effect. In these experiments the blood was drawn at the same operation from the carotid artery and from the jugular vein, and it would appear that the serum from the venous blood was somewhat more sensitive to the action of the heat than the serum from the arterial blood, if any conclusions are permissible from the somewhat limited number of observations. In the arterial there was in only one case a marked difference between the potency of the heated and of the unheated serum out of the 12 cases in which it was tried. In the serum from the venous blood, on the other hand, there was a marked difference between the heated and the unheated serum in the 11 tests which were made. On the whole the effect of heating at 52° C. would appear to be uncertain; sometimes heating seems to have a marked effect upon the bactericidal power of the serum; at others it seems to lack this effect.

TABLE XIV.—*Effects of heat upon the bacteriolytic power of normal hog serum. Blood drawn from the tail. Temperature employed, 53° C.*

Date.	Time after drawing.	No. of hog and drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.		Length of exposure to heat.
					Not heated.	Heated.	
1905.							
Apr. 19	1 day	1733 V	G. P. 4692	8,655	142,900	49,000	10 minutes.
19	do	do	*B. coli*	2,600	0	37,800	Do.
22	2 days	1733 VI	G. P. 4692	4,968	43,200	26,600	Do.
22	do	do	*B. coli*	1,940	240	2,100	Do.
26	1 day	1733 VII	G. P. 4692	1,800	1,220	2,988	Do.
26	do	do	*B. coli*	2,800	16,870	16,520	Do.
May 3	3 hours	1733 VIII	G. P. 4692	32,628	17,510	38,780	Do.
3	do	do	*B. coli*	23,380	84	9,920	Do.
5	do	1733 IX	G. P. 4692	8	1,004	13,720	Do.
5	do	do	*B. coli*	869	2,594	23,590	Do.
6	do	1740 I	G. P. 4692	4	1,750	89,600	Do.
6	do	do	*B. coli*	975	8	2,260	Do.
8	do	1740 II	G. P. 4692	4,925	1,276	7,380	Do.
8	do	do	*B. coli*	3,778	70	5,925	Do.
9	do	1740 III	G. P. 4692	102	1,236	1,900	Do.
10	do	1740 IV	G. P. 4692	1,010	1,552	3,559	Do.
10	do	do	*B. coli*	3,110	994	1,430	Do.
11	do	1740 V	G. P. 4692	848	450	2,354	Do.
12	do	1740 VI	G. P. 4692	138	0	1,550	Do.
12	do	do	*B. coli*	1,700	1,840	15,900	Do.
8	do	1741 I	G. P. 4692	4,925	82	4,144	Do.
8	do	do	*B. coli*	3,778	14	1,132	Do.
9	do	1741 II	G. P. 4692	102	166	910	Do.
9	do	do	*B. coli*	1,970	390	1,280	Do.
10	do	1741 III	G. P. 4692	1,010	1,932	1,778	Do.
10	do	do	*B. coli*	3,110	40	70	Do.
11	do	1741 IV	G. P. 4692	848	50	3,286	Do.
12	do	1741 V	*B. coli*	1,700	0	1,181	Do.
8	do	1742 I	G. P. 4692	4,925		7,830	Do.
8	do	do	*B. coli*	3,788	686	17,580	Do.
10	do	1742 II	G. P. 4692	102	46	[a] ∞	Do.
10	do	do	*B. coli*	1,970	82	734	Do.
12	do	1742 III	G. P. 4692	1,010	798	2,412	Do.
12	do	do	*B. coli*	3,110	30	27,220	Do.
11	do	1742 IV	G. P. 4692	848	50	1,758	Do.
13	do	1742 V	*B. coli*	1,700	0	1,181	Do.
July 12	1 day	1733 XI [b]	F. 26	5,420	10,480	∞	30 minutes.
13	2 days	do	do	6,520	20,160	14,880	Do.
12	1 day	do	Crawford	8,160	160	33,500	Do.
13	2 days	do	do	8,660	400	16,720	Do.
12	1 day	do	G. P. 4692	2,160	2,520	2,440	Do.
13	2 days	do	do	2,720	2,960	2,180	Do.
12	1 day	1733 XI [c]	F. 26	5,420	220	5,680	Do.
13	2 days	do	do	6,520	27,680	41,720	Do.
12	1 day	do	Crawford	8,160	10,860	26,160	Do.
13	2 days	do	do	8,660	360	15,600	Do.
12	1 day	do	G. P. 4692	2,160	3,300	2,240	Do.
13	2 days	do	do	2,720	3,340	(?)	Do.

[a] This character signifies that there were too many colonies to count.
[b] Arterial blood serum.
[c] Venous blood serum.

As shown by the results recorded in Table XIV, heating at 53° C. had the effect in most cases of greatly diminishing the bactericidal power of the serum, though this is not to be noted in all cases, even where the serum was exposed for thirty minutes.

TABLE XV.—*Effects of heat upon the bacteriolytic power of normal hog serum. Blood drawn from the tail. Temperature employed, 54° C.*

Date.	Time after drawing.	No. of hog and drawing of blood.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.		Length of exposure to heat.
					Not heated.	Heated.	
May 26	3 hours	1742 VIII	Crawford	320	0	1,160	10 minutes.
June 2	7 days	do	do	1,960	380	2,380	Do.
May 26	3 hours	do	F. 26	80	1,960	5,280	Do.
June 2	7 days	do	do	3,280	2,980	9,360	Do.
May 29	3 hours	1742 IX	do	1,740	4,480	13,600	Do.
June 3	5 days	do	do	1,220	1,180	1,180	Do.
May 29	3 hours	do	Crawford	2,320	0	9,520	Do.
June 3	5 days	do	do	1,060	0	1,880	Do.
May 29	3 hours	do	G. P. 4692	1,920	960	5,040	Do.
June 3	5 days	do	do	700	1,100	1,260	Do.
5	½ hour	1742 X	F. 26	2,840	26,640	119,700	Do.
6	1 day	do	do	2,240	51,760	41,840	Do.
7	2 days	do	do	3,440	900	6,440	Do.
8	3 days	do	do	2,200	1,340	7,280	Do.
9	4 days	do	do	1,360	18,280	20,480	Do.
19	14 days	do	do	4,040	[a] ∞	∞	Do.
5	½ hour	do	Crawford	3,380	180	28,000	Do.
6	1 day	do	do	5,200	120	8,320	Do.
7	2 days	do	do	7,440	760	13,000	Do.
8	3 days	do	do	3,500	3,160	48,000	Do.
9	4 days	do	do	2,640	1,380	∞	Do.
19	14 days	do	do	1,440	∞	∞	Do.
5	½ hour	do	G. P. 4692	1,380	4,420	4,820	Do.
6	1 day	do	do	3,040	6,320	27,760	Do.
7	2 days	do	do	180	3,420	∞	Do.
8	3 days	do	do	360	2,000	17,960	Do.
9	4 days	do	do	1,880	∞	∞	Do.
19	14 days	do	do	1,740	∞	∞	Do.
May 23	do	1742 VI	do	10,880	3,376	13,180	30 minutes.
23	do	do	Crawford	40,500	2,640	58,800	Do.
23	do	do	F. 26	19,040	18,280	∞	Do.
24	do	1742 VII	G. P. 4692	1,682	0	2,720	Do.
24	do	do	Crawford	1,808	180	7,600	Do.
24	do	do	F. 26	12,040	1,640	26,320	Do.

[a] This character signifies that there were too many colonies to count.

As is evident from the preceding table the effect of exposure for ten minutes at 54° C., as well as exposure for thirty minutes at the same temperature, is practically in all cases to weaken or suspend the bacteriolytic power of the serum. The few exceptions to be seen in the table are without significance in view of the many cases in which the serum lost in power.

In summing up the results of heat upon the bacteriolytic power of normal hog serum it would seem evident that temperatures below 54° C. are uncertain in action for the lengths of time employed, but that 54° C. even for ten minutes serves quite uniformly to suspend or at least to weaken the bacteriolytic power of the blood serum. A number of tests were made at 55° C. and 56° C., but as these did not show any results that were not to be expected from those at 54° C. it would seem superfluous to give them in detail.

EFFECTS OF INJECTING CULTURES OF B. CHOLERÆ SUIS UPON THE BACTERIOLYTIC POWER OF HOG SERUM FOR THIS ORGANISM.

Very conflicting results have been obtained by various investigators in regard to the effects produced upon the bacteriolytic power of blood serum by the injection of animals with cultures of bacteria. Some have failed to note any effect of such injections, either to increase or diminish the bacteriolytic potency; others have found that the potency was diminished, others again that it was increased. Under the supposition that this discrepancy in results was due perhaps to the tests having been made at different intervals of time after injection, and that the serum taken from the same animal at different periods might exhibit differences in potency, or that it might at certain times be devoid of power, tests were made of serum taken at various intervals after one or more injections. The results of these experiments are summarized in the following tables.

TABLE XVI.—*Bacteriolytic power of serum from the same hog (No. 1754) before and after injection with B. choleræ suis, culture G. P. 4692.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection	....	....
Do	3 days after first injection	2,180	260
Do	9 days after second injection	240	0
Do	10 days after third injection	5,200	0
F. 26	Before injection	....	....
Do	3 days after first injection	16,700	11,800
Do	9 days after second injection	7,600	2,640
Do	10 days after third injection	5,200	26,700
G. P. 4692	Before injection	1,060	3,300
Do	3 days after first injection	638,400	12,800
Do	9 days after second injection	6,340	1,020
Do	10 days after third injection	4,100	16,700

The results given in Table XVI were obtained with the serum from a hog which was injected at various intervals subcutaneously with a beef-broth suspension from an agar culture of the G. P. 4692 strain.

The first injection was made with 5 c. c. of a suspension equivalent in density to a twenty-four-hour typhoid culture diluted about one-half. The second injection was made with 10 c. c. of a suspension of about the same strength. The third injection was made with a suspension consisting of the entire growth in twenty-four hours of an agar culture. Before each injection the blood was drawn from the tail of the animal and placed on ice for three days. After this the serum was drawn off from the blood and tested on the three strains in all cases except the serum from the blood taken at the first drawing, which was tested only upon the culture with which the animal was subsequently injected—culture G. P. 4692.

As there were no tests made with culture Crawford before the animal was injected, it is impossible to be sure whether it was bactericidal for this strain at this time or not, but judging from its action on G. P. 4692 before injection it would seem probable that it would have been bactericidal or at least inhibitory for culture Crawford, since the latter strain was quite uniformly more sensitive to the germicidal action of serum in other experiments. The serum after the injections possessed quite strong bacteriolytic properties for culture Crawford.

It is also possible only to surmise from the results with G. P. 4692 that the serum probably had at least inhibitory power for F. 26 before the animal was injected. It seems to have retained its inhibitory power after the first injections for this organism, but to have lost much in potency after the third injection. With culture G. P. 4692 the results seem to be more or less in accord with those obtained with F. 26, though it is true that three days after the first injection the serum exhibited very strong bactericidal power for G. P. 4692—stronger than for F. 26. Still the results can not be compared too closely in these two cases, since the number of bacteria introduced differed widely.

TABLE XVII.—*Bacteriolytic power of serum from the same hog (No. 1755) before and after injection with B. choleræ suis, culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection	...	...
Do	3 days after first injection	2,180	40
Do	9 days after second injection	240	0
Do	10 days after third injection	5,200	0
F. 26	Before injection	800	47,400
Do	3 days after first injection	16,700	380
Do	9 days after second injection	7,600	11,880
Do	10 days after third injection	8,200	660
G. P. 4692	Before injection	...	...
Do	3 days after first injection	638,400	9,800
Do	9 days after second injection	6,340	3,800
Do	10 days after third injection	4,100	11,200

The results of the experiments with the serum of hog 1755 are recorded in Table XVII. In this experiment the injections were made and the tests applied precisely as with hog 1754, except that culture F. 26 was used for injecting the animal instead of culture G. P. 4692. The concentration and amounts of culture were the same in both experiments, and the methods of procedure otherwise were the same. The serum from the hog before injection was tested upon the F. 26 strain only; after injection it was tested with all three.

For the Crawford culture, as already stated, there was no test of the serum made before the injection of the animal. The tests made with this organism after each of the three injections showed that the serum possessed considerable potency, and there seems no evidence that the injections were followed by diminution of potency for this organism.

For F. 26 culture the serum seems to have had but little potency to start with before injection, but it seems rather to have gained than lost in potency for this organism after injection.

For G. P. 4692 culture the results of the three tests are somewhat difficult of comparison, perhaps, but the serum after the third injection would appear to have been somewhat less potent than that after the second, and, also, after the first, if the difference in the initial doses in these two cases may be disregarded as a factor in determining the results.

TABLE XVIII.—*Bacteriolytic power of serum from the same hog (No. 1756) before and after injection with B. choleræ suis, culture Crawford.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection	700	0
Do	3 days after first injection	400	80
Do	9 days after second injection	240	0
Do	10 days after third injection	5,200	350
F. 26	Before injection	....	....
Do	3 days after first injection	16,700	2,280
Do	9 days after second injection	7,600	25,000
Do	10 days after third injection	8,200	22,700
G. P. 4692	Before injection	....	....
Do	3 days after first injection	638,400	5,960
Do	9 days after second injection	6,340	0
Do	10 days after third injection	4,100	8,300

In the experiment with the serum of the blood of hog 1756, the results of which are given in Table XVIII, the method of procedure was the same as with the serum of hogs 1754 and 1755, just described, the only difference being in the strain of organism employed. In the case of hog 1756 the Crawford culture was used, and the serum was tested upon this organism only before injection and on all three strains after the injections.

With the Crawford culture the serum seems to have been unaffected by the injections of the hog. It seems, at any rate, not to have lost in potency, and from the result after the second injection, if any conclusion is justifiable, it seems possibly to have gained.

With F. 26 it seems to have lost in potency, if the result obtained after the first injection is compared with those after the other two.

With G. P. 4692 culture the serum seems to have lost in potency after the third injection, but it is uncertain whether it gained or lost after the first two.

TABLE XIX.—*Bacteriolytic power of serum from the same hog (No. 1798) before and after injection with B. choleræ suis, culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
F. 26	Before injection	2,620	1,980
Do	10 days after first injection	8,400	7,200
Do	9 days after second injection	5,920	38,000
Do	14 days after third injection	2,400	36,600

In the experiment with the serum of hog 1798, Table XIX, the animal was given three intravenous injections of suspensions in beef broth from agar cultures of the F. 26 strain. The first one was made with a very dilute suspension, weaker than would correspond with a twenty-four hour beef-broth typhoid culture, and the other two injections were made with similar suspensions.

It would appear from the results given in the table that the serum was not actively bactericidal before the injection of the animal, but it seems to have had some inhibitory power. Ten days after the first injection there seems to have been no striking change. The serum was still inhibitory, but was neither markedly bactericidal nor can it be said to have been devoid of potency. But nine days after the second injection, and fourteen days after the third injection, the serum seems to have lost greatly in the inhibitory power which it possessed. It would appear that in this case the injections had the effect of ultimately diminishing the power of the serum.

Table XX.—*Bacteriolytic power of serum from the same hog (No. 1809) before and after injection with B. choleræ suis, culture G. P. 4692.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
F. 26	Before injection	5,920	5,900
Do	14 days after injection	7,300	1,800
Crawford	Before injection	5,700	0
Do	14 days after injection	13,000	56,600
G. P. 4692	Before injection	1,140	10,900
Do	14 days after injection	7,300	7,900

In the experiment given in Table XX the serum of hog 1809 was tested on the three different strains of *B. choleræ suis* before the animal was injected and on the same cultures fourteen days after the hog had received subcutaneously about 5 c. c. of a dilute suspension from an agar culture of culture G. P. 4692. In the tests made with the serum upon the F. 26 strain there seems to have been a slight diminution of potency after the inoculation, but the difference in the numbers of bacteria surviving bacteriolysis in the serum from the hog before injection and those surviving after injection is not large enough to permit of very definite conclusions. The same may be said in regard to the organism with which the hog was injected—culture G. P. 4692—but the results in this case are perhaps somewhat more significant. It would seem justifiable, however, to conclude that there was no marked increase nor decrease of bacteriolytic power for F. 26 culture or for G. P. 4692 produced by the injection. The result with Crawford seems to show plainly that the serum was less potent for this organism after injection than it was before.

Table XXI.—*Bacteriolytic power of serum from the same hog (No. 1836) before and after injection with B. choleræ suis, culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection	32,200	[a] 3,840
Do	1 hour after injection	32,200	[a] 5,340
Do	3 days after injection	1,560	[b] ∞
F. 26	Before injection	30,400	[a] 247,800
Do	1 hour after injection	30,400	[b] ∞
Do	3 days after injection	5,000	∞
G. P. 4692	Before injection	35,000	[a] 47,400
Do	1 hour after injection	35,000	[a] 36,600
Do	3 days after injection	6,800	∞

[a] Serum diluted in the proportion of 1 : 4.

[b] This character signifies that there were too many colonies to count.

In the experiment with the serum from the blood of hog 1836, given in Table XXI, the animal was injected intravenously with a suspension from an agar culture of strain F. 26 of about the density of a 24-hour beef-broth culture of the typhoid bacillus. Before injection the hog was bled to obtain serum for testing the power of the normal serum. The amount of blood obtained before injection and that obtained one hour after injection was insufficient for the test to be made with the quantity of serum usually employed, so the serum was diluted in both of these cases with three parts of physiological salt solution to one part of serum. There were no tests made with the undiluted serum.

With the Crawford culture the serum seems to have been quite potent before injection, and also one hour after injection, but three days after injection it appears to have been entirely devoid of bacteriolytic power for this organism.

With F. 26—the strain used to inject the animal—the serum had only weak potency before injection, and if there was any effect produced by the injection it was in the direction of weakening the potency of the serum for this organism. This apparent diminution of potency is to be noted even in one hour after the injection, and is still more pronounced after three days.

With the G. P. 4692 strain the serum was apparently inhibitory at the start before injection and one hour afterwards, but three days after injection it seems to have lost even the power to inhibit the growth of the organism.

The results of this experiment seem to show that in this case the effect of injecting the hog was to suspend the bactericidal power of the serum for the homologous[a] organism as well as for the other strains upon which it was tested.

TABLE XXII.—*Bacteriolytic power of serum from the same hog (No. 1859) before and after injection with B. choleræ suis, culture G. P. 4692.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection	3,760	80
Do	2 hours after injection	3,760	1,460
Do	1 day after injection	600	266,400
Do	2 days after injection	600	18,000
F. 26	Before injection	1,020	2,900
Do	2 hours after injection	1,020	80
Do	1 day after injection	340	39,000
Do	2 days after injection	340	36,400
G. P. 4692	Before injection	2,840	3,800
Do	2 hours after injection	2,840	9,200
Do	1 day after injection	200	54,600
Do	2 days after injection	200	12,400

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[a] Homologous is the term now universally employed to denote the organism with which the animal in any given case is injected, as in the present case.

In the experiment given in Table XXII the animal—hog 1859—was injected intravenously with 5 c. c. of a suspension of culture G. P. 4692. The suspension was made in beef broth from a 24-hour agar culture as usual, and was equivalent in density to a 24-hour beef-broth culture of the typhoid culture diluted with about equal amounts of broth. Tests were made of the potency of the serum from the blood of the animal before injection and after injection with all three strains of *B. choleræ suis.* Owing to the fact that the animal was killed in a moribund condition two days after injection, it was impossible to make tests with the serum from the blood later than the second day after injection.

It would seem hardly necessary to discuss the results with the different organisms in detail, since they all appear to point to a lessening of the potency one and two days after injection. Before injection the serum from the blood seems to have been bactericidal for the Crawford strain only; still for the other strains it was at least inhibitory, whereas after injection it had lost much, if not all, even of its inhibitory power on the first and second day after injection. In the tests made two hours after injection the results appear somewhat discrepant; the serum seems to have lost potency for Crawford, and gained for F. 26; but, after all, the figures are perhaps not very significant in these cases.

The results in this experiment may have been complicated by the fact that the animal from which the blood was drawn was suffering from acute infection with *B. choleræ suis* at the time the blood was obtained. It may not be correct to regard this serum as immune in the sense in which this term is usually understood—that is to say, serum from the blood of an animal given injection of bacteria and recovered. Nevertheless, the results are perhaps not without interest, since they show certainly a very marked difference between the serum from the blood of the hog before injection and that from the blood after injection.

TABLE XXIII.—*Bacteriolytic power of serum from the same hog (No. 1860) before and after injection with B. choleræ suis, culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria per 1 c. c. after 1 day.
Crawford	Before injection	1,460	0
Do	1 day after injection	1,240	20
Do	2 days after injection	140	[a] ∞
Do	3 days after injection	5,400	∞
Do	4 days after injection	1,620	0
F. 26	Before injection	2,100	80
Do	1 day after injection	700	880
Do	2 days after injection	1,100	∞
Do	3 days after injection	1,500	∞
Do	4 days after injection	2,260	5,580
G. P. 4692	Before injection	10,200	17,400
Do	1 day after injection	4,100	60
Do	2 days after injection	1,500	∞
Do	3 days after injection	9,900	∞
Do	4 days after injection	1,380	1,020

[a] This character signifies that there were too many colonies to count.

In the experiment recorded in Table XXIII, the animal—hog 1860—was injected first with about 2 c. c. of a dilute suspension of an agar culture in beef broth, but most of the material was injected merely under the skin, as the attempt to strike a vein was not successful. Two days after the first injection a second injection of about 2.5 c. c. of a similar suspension from the same strain of bacterium, in this case all of the suspension, was introduced into the vein. Two days after the second injection a third injection was made similar in all respects to the second. The suspensions for all three of the injections were heated at 57° to 58° C. for thirty minutes. The blood was drawn from the animal before injection and on the first, second, third, and fourth days after the last injection. The results seem to be quite uniform for the serum on the second and third days after the injections; on both days the serum showed apparently entire loss of bactericidal power for all three organisms. On the fourth day after the last injection the serum had apparently gained in potency, particularly for the Crawford strain. The results obtained with the serum one day after the last injection are not very striking, but they perhaps permit of the conclusion that the serum had not lost in potency at least to any marked extent.

As previously stated, the results of the injection of animals with cultures or with the products of growth of bacteria have been most conflicting in various hands in so far as the effect of such injections upon the bacteriolytic properties of the blood serum from the animal in test-tube experiments is concerned.[7]

A number of investigators have found that the injection of an animal with cultures or with the products of growth of certain bacteria increases the bactericidal power of the blood serum for the homologous organism. Others have found that there is no effect upon the bactericidal potency of the serum produced by such injections, while still others have found diminution or complete loss of power for the homologous organism to follow the injections. Among others Neisser and Wechsberg[14] have found that the so-called immune serum—i. e., the serum from an animal which has received injections of certain bacteria—no longer possesses bactericidal power for the homologous organism when the serum is undiluted. The results with the diluted serum will be discussed later. Buxton[4] has found that the serum from the blood of a normal rabbit is capable of destroying very large numbers of the typhoid and paratyphoid bacilli, but that after the rabbit has been injected with these organisms it no longer kills any of the homologous strain.

But while there is undoubtedly conflict in regard to the matter, the preponderance of evidence certainly seems to show that the serum is frequently, if not always, weakened in bactericidal power or completely robbed of this power for the homologous organism by these injections, and the results recorded above seem in many instances to bear out this result in the case of hogs injected with *B. choleræ suis.* But it would also seem apparent that there is a tendency in some cases to a restoration of the lost potency on further injection of the animal.

These conclusions seem justifiable at least from the experiments with the F. 26 and the G. P. 4692 strains. The results with the Crawford strain differ somewhat in this respect from those with the other two strains, and this may be due to the fact, already mentioned, that this organism is of only feeble pathogenic potency. It seemed to be much more easily killed by the serum generally than the other two strains, and it probably also has less power than these to cause the reaction in the serum which is necessary for the production of immune serum as this is understood. The serum of hogs injected with this strain would consequently perhaps be expected to behave more like the serum from an uninoculated animal. In some cases the serum of the blood of hogs injected with the F. 26 or with the G. P. 4692 strain seemed to have retained bactericidal power for the Crawford strain after having lost it for the other two.

The variations which have been already alluded to as occurring in the serum of animals from time to time, and which are due to causes as yet obscure, must be borne in mind in drawing conclusions from the results of experiments with the serum from animals injected with cultures. Of course it is impossible to determine in the present case the extent to which this spontaneous variation, as it is at present convenient to call it, may have affected the results.

In view of the many conflicting results with the serum of the blood both of normal and of injected animals, in so far as the bactericidal properties are concerned, it is evident that there are some obscure factors in the process which the methods at present in use are incapable of determining. If the blood serum from one and the same animal is found to be bactericidal at one time and not at another, where the animal is kept under apparently the same conditions, and if, furthermore, the injection of cultures is found by certain observers to have no effect upon the bactericidal potency of the serum, by others to increase the potency, and by others again to lessen the potency, there must be some very subtle factors which enter into the problem, else they would scarcely be overlooked by so many investigators.

EFFECTS OF DILUTION UPON THE BACTERIOLYTIC POWER OF NORMAL AND IMMUNE SERUM.

DILUTION OF NORMAL SERUM.

The effects of dilution upon the bactericidal power of hog serum were tested in the manner usually employed in such cases. The method for obtaining the blood was to cut off a piece of the tail and allow the blood to run into sterile test tubes or flasks, which were put into the refrigerator immediately. The serum was drawn off as soon as it had separated, and 1 c.c. of it was put into each of three tubes. Various dilutions, as mentioned in the tables, were also prepared with physiological salt solution, and portions of these dilutions were distributed into test tubes, three tubes from each dilution, each containing 1 c.c. In most cases 0.1 or 0.05 c.c. of neutral, peptonized beef broth was added to insure sufficient nutrient material for the bacteria which were subsequently introduced. This was done in conformity with the method ordinarily employed in such experiments. Each of the tubes was finally inoculated with a carefully measured amount, usually 0.05 c.c., of a suspension in physiological salt solution from a recent agar culture. One series each was inoculated with one of the three strains of *B. choleræ suis*. In addition to the tubes of undiluted and of diluted serum, three tubes of physiological salt solution with the addition of 0.05 c.c. of beef broth were also prepared in each case, and inoculated at the same time as the serum tubes with an amount of culture equal to that used for the serum tubes. These tubes constituted the checks. Immediately after the inoculation of the tubes, agar plates were made from each of the tubes of salt solution with carefully measured amounts, and after twenty-four hours at room temperature agar plates were made from all the tubes of a series—from both the undiluted and diluted serum, and from the salt solution. In nearly all cases 0.05 c. c. was used for each plate. The colonies were counted after twenty-four or forty-eight hours in the incubator at body temperature.

TABLE XXIV.—*Bacteriolytic power of normal hog serum on dilution.*

No. of hog.	Culture used to test bacteriolysis.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 50.	Diluted 1 to 500.	Diluted 1 to 5,000.	
1798	Crawford	840	0	26,400	81,200	140	....
	F. 26	2,620	1,980	1,400	1,740	2,280	....
	G. P. 4692	2,660	1,680	2,700	32,800	5,200	....
1808	Crawford	840	0	0	74,200	2,340	....
	F. 26	8,400	2,040	35,200	[a]∞	161,000	....
	G. P. 4692	2,060	2,900	2,980	1,980	900	....
1809	Crawford	5,700	0	520	∞	∞	∞
	F. 26	5,920	5,900	109,200	∞	∞	∞
	G. P. 4692	1,140	10,900	2,880	123,200	60,800	73,000
				Diluted 1 to 10.	Diluted 1 to 100.	Diluted 1 to 1,000.	
1836	Crawford	32,200	[b]3,840	320	135,800	∞	∞
	F. 26	30,400	[b]247,800	117,600	∞	∞	∞
	G. P. 4692	35,000	[b]47,400	37,200	308,000	∞	∞
1859	Crawford	3,760	80	1,160	149,800	∞	∞
	F. 26	1,020	2,900	13,000	187,600	∞	∞
	G. P. 4692	2,840	3,800	6,200	16,080	∞	∞
1860	Crawford	1,460	0	80	12,500	118,400	18,700
	F. 26	2,100	80	30,480	504,600	879,200	∞
	G. P. 4692	10,200	17,400	11,400	61,400	445,200	206,000

[a] This character signifies that there were too many colonies to count.

[b] Diluted 1 to 4.

The results given in Table XXIV show that the serum taken from a hog before injection—i. e., normal serum—as a rule, loses in potency upon dilution; that is to say, a given amount of the diluted serum kills actually fewer bacteria than the same amount of the undiluted serum. This was to be expected a priori and from the results of others with other organisms and with other animals. But the point which seems specially worthy of note, and which will be discussed in a different connection in this paper, is that the diminution of potency on dilution is not in proportion to the degree of dilution. Not only is this the case, but in at least 4 out of the 18 experiments given in the table there was apparently an actual increase of power on dilution. Those familiar with the subject will at once see the bearing of these results upon the difference between the bacteriolytic properties of normal serum upon the one hand and of immune serum on the other. As it is attempted to show below, the difference seems, after all, to be one of degree only, and not a difference of kind.

In interpreting the above results it must of course be borne in mind that the number of bacteria found in the serum after twenty-four hours probably does not represent merely the bacteria which are not killed by the serum, but it represents in addition the number resulting from the multiplication of these after bacteriolysis has ceased. Thus, in the case of the serum from hog 1859, if 1 c. c. killed approximately 4,000 of the Crawford bacilli, 0.1 c. c. of the serum should evidently have killed 400 and have left 3,600 to multiply, unless in addition to the bactericidal power the serum also possessed inhibitory power for the bacteria which survived bacteriolysis. If the serum had exhausted its bacteriolytic power in ten or twelve hours and exerted no further influence after this time, there should have been in the 1 to 10 dilution after twenty-four hours a multiplication of the 3,600 bacilli left, resulting in some 4,000,000 or 5,000,000 even at the rate of only one generation an hour, as a simple calculation shows. But even if only one bacillus were left after twelve hours, there would result over 4,000 in twenty-four hours, provided there were no hindrance to multiplication at the rate assumed above. It would seem, therefore, that either relatively more bacilli were destroyed by the diluted serum than by the undiluted, or that, aside from the bacteriolytic action, the diluted serum exerted an inhibitory effect in the experiment quoted; for at the end of twenty-four hours there were only about 1,000 bacilli in the 1 to 10 dilution instead of the 4,000 calculated as the minimum number possible under the supposition stated.

The dilution of 1 to 1,000 in the same experiment should evidently have killed only 40 bacilli if it acted with the same relative intensity as the undiluted, i. e., if it possessed just one one-thousandth of the potency of the undiluted serum. Supposing that all bacteriolytic action ceased after fourteen hours, the 3,960 bacilli left alive increasing at the rate of one generation an hour would be represented by over 4,000,000 bacilli in ten hours, whereas as a matter of fact there were only about 32,000 in this dilution after twenty-four hours.

The time at which bacteriolysis is exhausted in serum at room temperatures was not determined, but tests made at the end of three or four hours showed that up to this time there was little or no appreciable effect upon the number of bacteria introduced, so that the above assumption that the process is exhausted in twelve or fourteen hours may or may not be correct. But whether it is or is not, the relative effect of the various dilutions would be shown by assuming the process to come to an end simultaneously—and this is probably the case—at any time within the twenty-four hours, the object of the calculation being merely to show that no definite rule of proportion could be observed, and that the diluted serum in all cases destroyed relatively more bacteria than the undiluted serum. While dilution of normal serum causes a marked diminution in the bactericidal power, it does not seem to diminish it to the extent that would be expected a priori. In other words, a dilution of 1 to 10 does not seem to destroy just one-tenth the number of bacilli that the undiluted serum destroys; but a much larger number than this, or at least if it does not destroy a larger number it prevents as relatively great an increase as that which takes place in the undiluted serum.

Still another method of comparison which appears permissible, and which leads to the same conclusion, is to calculate the number of bacteria which should result in a dilution of 1 to 100 if the same relative increase took place as in the 1 to 10 dilution; or to compare the relative increase in any of the dilutions with that in the undiluted serum in the experiment given above. For such a comparison it may be assumed that the organisms which were not destroyed in fourteen hours multiplied in the undiluted and in the diluted serum at the same rate, or at least that if the serum retarded multiplication this effect was more marked in the more concentrated serum than in the more dilute. It would seem evident, then, that the relatively smaller number of bacteria in the diluted serum can be accounted for only on the supposition that the diluted serum retains more bacteriolytic power than would be expected from the degree of potency shown by the undiluted serum, according to the simple rule of proportion. This point seems not to have been alluded to in the literature, but it would seem nevertheless not without significance perhaps, since it tends apparently to show that the difference between normal serum on the one hand and immune serum on the other is merely a difference of degree and not a difference of kind. The matter of the actual identity in the behavior of normal sera and of immune sera on dilution, in spite of the apparent difference shown by them, will be discussed more fully in a different connection below, where the attempt is made to show that the law of complement diversion applies to normal serum as well as to immune serum.

DILUTION OF IMMUNE SERUM.

The results obtained by tests made to determine the effect of dilution upon the bacteriolytic power of immune hog serum—that is to say, of serum from hogs injected with cultures—in the main agree with those observed in all recent investigations in this direction with the serum of other animals injected with typhoid, paratyphoid, Asiatic cholera, and other bacteria. The behavior of the serum under such conditions is very peculiar; it seems to be in conflict with all laws of proportion which are applicable to other chemical reagents, particularly in regard to germicidal substances in general. With germicidal substances in general, as it is hardly necessary to state, the more concentrated the germicide the more powerful the destructive power. A strong solution of carbolic acid is a more powerful germicide than a weaker solution. But not so with immune blood serum. Paradoxical as it may seem, the bacteriolytic potency of the immune serum increases upon dilution. The undiluted immune serum may be weak or wanting in bactericidal properties, and yet become more and more potent upon dilution. In other words, 1 c. c. of undiluted immune serum may be found to kill few, if any, bacteria; it may be found even to furnish a good nutrient medium in which the bacteria grow; whereas 1 c. c. of a dilution consisting of 1 part of the same serum to 10 or 100 or 1,000 parts of dilute salt solution may be found to kill many hundred bacteria introduced into it. It goes without saying, of course, that there is a limit to this increase of potency upon dilution. Where the dilution goes beyond a certain point the potency becomes weakened. There is for each immune serum an optimum dilution for bacteriolysis, above and below which there is less and less potency. Thus, as will be seen in some of the experiments described below, the greatest potency in one case was in a dilution of 1 part of serum to 100 parts of salt solution. In the case of a different serum the optimum dilution for bacteriolysis was 1 part of serum to 1,000 parts of salt solution.

Buxton very appropriately speaks of the phenomenon just described as the formation of bacteriolytic zones, of which three may be recognized in every immune serum—a pro-killing zone, a killing zone, and a post-killing zone. The action of the undiluted immune serum lies in the pro-killing zone, that of the optimum dilution lies in the killing zone; that of dilutions in which bacteriolysis becomes again weakened lies in the post-killing zone. Of course it is to be understood that these zones are not sharply marked off from one another, but that they gradually merge into each other. The pro-killing zone, for example, may extend over dilutions of several strengths, and the same is true of the killing and of the post-killing zones. Thus in some of the experiments given below there appeared to be no marked difference between the bactericidal power of the undiluted serum and the same serum diluted in the proportion of 1 to 10 or 1 to 100; the pro-killing zone in these cases would be said to extend to the dilutions of 1 to 10 or 1 to 100. The zones are therefore spoken of as longer or shorter, according to whether they apply to many or to few dilutions.

The peculiar behavior of immune serum just described was first observed by Neisser and Wechsberg,[14] and is called the Neisser-Wechsberg phenomenon. While it is true that this phenomenon was observed quite frequently in immune hog serum, as will be shown later, it was not as noticeable in some cases as it was in others, and moreover the three zones varied in length, not only after the injection of different strains of *B. choleræ suis* in different hogs, but also after the injection of the same strain in different hogs.

It has already been shown that normal serum of hogs gains relatively in potency on dilution, and, as has just been stated, immune serum also gains in potency. The two behave alike, and at most there is only a difference of degree between the amount of gain in either case. But even a difference of degree between the two sorts of serum is not always noticed in the present experiments, for the normal serum acted in some cases just like immune serum. It was not only relatively more potent on dilution, but it killed actually more bacteria per 1 c. c. of serum in certain dilutions than in dilutions containing more of the serum.

TABLE XXV.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1859, injected with culture G. P. 4692.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 10.	Diluted 1 to 100.	Diluted 1 to 1,000.	
Crawford	Before injection	3,760	80	1,160	149,800	[a] ∞	∞
	2 hours after injection	3,760	1,460	200	4,900	∞	∞
	1 day after injection	600	266,400	4,500	80	520	1,580?
	2 days after injection	600	18,000	700	4,500	12,400	1,580?
F. 26	Before injection	1,020	2,900	13,000	187,600	∞	∞
	2 hours after injection	1,020	80	11,400	162,400	∞	∞
	1 day after injection	340	39,000	27,800	480	5,500	2,700?
	2 days after injection	340	36,400	1,800	9,440	900	2,700?
G. P. 4692	Before injection	2,840	3,800	6,200	16,080	∞	∞
	2 hours after injection	2,840	9,200	27,400	15,200	∞	∞
	1 day after injection	200	54,600	22,400	19,200	60	200?
	2 days after injection	200	12,400	140	400	1,600	200?

[a] This character signifies that there were too many colonies to count.

An example of the difference often to be seen between the bactericidal power of the serum from a hog before and after injection with cultures of *B. choleræ suis*, in so far as the effect of dilution is concerned, is given in Table XXV. The animal in this case was injected with a culture of the G. P. 4692 strain, and the blood was drawn before and at several intervals after injection. Before the injection the serum from the blood of this hog showed inhibitory, but no bactericidal power for the organism with which the animal was injected.

In this case there were 2,840 of these organisms introduced per 1 c. c. into the undiluted serum, and after twenty-four hours there were 3,800 per 1 c. c. present. In the dilution of 1 to 10 the same number of organisms introduced about doubled in number in twenty-four hours; in the dilution of 1 to 100 the same number of organisms increased about fivefold; in the 1 to 1,000 dilution there was a countless increase. In the undiluted serum from the blood of the same hog two days after inoculation there was a sixty-two fold increase of the 200 bacilli per 1 c. c. which were introduced, while in the 1 to 10 dilution with the same number of bacilli introduced there were only 140 alive after twenty-four hours, in the 1 to 100 dilution only 400, and in the 1 to 1,000 there were 1,600. In other words, the normal serum diminished in potency on dilution while the immune serum increased in potency on dilution. It should be noticed, however, that the diminution of potency of the normal serum is an actual and not a relative decrease on dilution. In this case, as in others, the normal serum killed more bacteria, relatively, when it was diluted than it did when undiluted.

TABLE XXVI.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1798, injected with culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 50.	Diluted 1 to 500.	Diluted 1 to 5,000.	
F. 26	Before injection	2,620	1,980	1,400	1,740	2,280	....
	10 days after first injection.	8,400	7,200	106,400	103,600	6,900	....
	9 days after second injection.	5,920	38,000	38,400	84,000	[a] ∞	∞
	14 days after third injection.	2,400	36,600	[b] 86,800	[b] 81,200	[b] 112,000	∞
Crawford	10 days after first injection.	840	0	26,400	81,200	140	....
	9 days after second injection.	5,700	160	400	27,600	∞	∞
	14 days after third injection.	13,000	359,600	[b] 12,000	[b] 20,400	[b] 175,000	∞
G. P. 4692	10 days after first injection.	2,660	1,680	2,700	32,800	5,200	....
	9 days after second injection.	1,140	1,600	2,000	5,500	26,600	73,000
	14 days after third injection.	7,300	5,100	[b] 9,100	[b] 7,500	[b] ∞	∞

[a] This character signifies that there were too many colonies to count.
[b] Dilutions used were 1 to 10, 1 to 100, 1 to 1,000.

That there is a difference in the behavior of the blood serum of different hogs injected with the same strain of *B. choleræ suis* is shown by comparing the results given in Table XXVI, hog 1798, with those shown in Table XXVII, hog 1860. Both of the animals were injected with culture F. 26. Before injection the blood of hog 1798 showed considerably more bacteriolytic power on dilution for the organism with which the animal was afterwards injected than the blood serum of hogs did in other cases, though, as has been seen, normal hog serum frequently retains a considerable degree of potency upon dilution. This serum appears to have possessed about an equal degree of potency in all the dilutions which were tried, and to have been about as potent when diluted as it was when undiluted. Ten days after injection its behavior was very peculiar, for it was apparently about as potent as the normal serum when it was undiluted and in the 1 to 5,000 dilution, but it had lost potency in the dilutions of 1 to 50 and 1 to 500. Nine days after a second injection it had apparently lost in bactericidal potency, both when diluted and undiluted. Fourteen days after a third injection it seems to have gained in power in all strengths. If this is compared with the behavior of the serum from the blood of hog 1860, Table XXVII, a hog also injected with the F. 26 strain, it will be noticed that there is a marked difference.

TABLE XXVII.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1860, injected intravenously on four successive days with culture F. 26, about 5 c. c. heated for thirty minutes at 58–59° C.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 10.	Diluted 1 to 100.	Diluted 1 to 1,000.	
Crawford	Before injection	1,460	0	80	12,500	118,400	18,700
	1 day after injection	1,240	20	14,800	2,220	5,200	5,760
	2 days after injection	140	[a]∞	∞	19,000	6,600	460
	3 days after injection	5,400	∞	[b]∞	[b]∞	[b]∞	∞
	4 days after injection	1,620	0	0	2,300	14,400	20,400
F. 26	Before injection	2,100	80	30,480	504,600	879,200	∞
	1 day after injection	700	880	2,160	70,000	98,000	10,000
	2 days after injection	1,100	∞	∞	200,200	156,800	64,400
	3 days after injection	1,500	∞	[b]∞	[b]∞	[b]∞	∞
	4 days after injection	2,260	5,580	1,820	2,860	5,900	13,600
G. P. 4692	Before injection	10,200	17,400	11,400	61,400	445,200	206,000
	1 day after injection	4,100	60	8,800	10,600	98,000	28,000
	2 days after injection	1,500	∞	∞	64,400	46,800	4,400
	3 days after injection	9,900	∞	[b]∞	[b]∞	[b]∞	∞
	4 days after injection	1,380	1,020	2,100	2,480	10,800	∞

[a] This character signifies that there were too many colonies to count.
[b] Dilutions used were 1 to 50, 1 to 500, 1 to 5,000.

In the experiment with the blood serum of hog 1860, Table XXVII, the animal was injected on four successive days with quite dense suspensions, considerably denser than a twenty-four-hour culture of typhoid. The suspensions were heated for thirty minutes at 58–59° C. before injection, and the injections were made intravenously. On each day before injection, and previous to any injection, blood was drawn from the tail of the animal, and after the separation of the serum this was used on the three strains of *B. choleræ suis.* Doubtless the fact that the tests were made while the animal was being immunized had some effect upon the results obtained, and for this reason they are different in some measure from those obtained in other experiments in which the serum was tested only after the animal had been immunized.

The undiluted serum from this hog before injection was quite strongly bactericidal for the Crawford and the F. 26 strains, but it was at most merely inhibitory for the G. P. 4692 strain. After the second and third injections the serum seems to have lost all potency for all three organisms, at least when undiluted. But after the fourth injection the serum seems to have regained its potency both when undiluted and in certain of the dilutions.

TABLE XXVIII.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1835, injected with Crawford culture.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 10.	Diluted 1 to 100.	Diluted 1 to 1,000.	
Crawford ..	1 hour after injection ..	5,860	18,200	0	33,600	[a] ∞	∞
	1 day after injection....	3,200	71,100	5,200	11,000	∞	∞
F. 26.......	1 hour after injection ..	16,600	∞	110,400	∞	∞	102,200
	1 day after injection....	6,900	0	40,400	∞	∞	∞
G. P. 4692..	1 hour after injection ..	9,600	31,000	7,200	28,000	∞	∞
	1 day after injection....	4,600	3,600	96,000	10,700	∞	1,280?

[a] This character signifies that there were too many colonies to count.

In the experiment of which Table XXVIII gives the summary the hog was injected intravenously with a dilute suspension of the Crawford culture. Two injections of the organism were given on two consecutive days, and blood was drawn from the animal one hour and twenty-four hours after the last injection. No blood was drawn before injection in this case.

There seems to have been no difference between the behavior of the blood on dilution and that of ordinary normal blood on dilution. Omitting one or two unimportant discrepancies, the serum lost more and more in potency upon further and further dilution.

TABLE XXIX.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1809, injected with culture G. P. 4692.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in checksalt solution.
			Undiluted.	Diluted 1 to 50.	Diluted 1 to 500.	Diluted 1 to 5,000.	
F. 26.......	Before injection........	5,920	5,900	109,200	[a] ∞	∞	∞
	14 days after injection..	2,400	1,800	[b] 168,000	[b] 224,000	[b] ∞	∞
Crawford ..	Before injection........	5,700	0	520	∞	∞	∞
	14 days after injection..	13,000	56,600	[b] 4,000	[b] 6,600	[b] ∞	∞
G. P. 4692 ..	Before injection........	1,140	10,900	2,880	123,200	60,800	73,000
	14 days after injection..	7,300	7,900	[b] 9,200	[b] 25,900	[b] ∞	∞

[a] This character signifies that there were too many colonies to count.
[b] Diluted in the proportion of 1 to 10, 1 to 100, 1 to 1,000.

In the experiment summarized in Table XXIX the hog was injected subcutaneously with 5 c. c. of a suspension of G. P. 4692, but there seems to have been little if any effect produced upon the bacteriolytic power of the serum. It is true that with the Crawford strain there is some evidence of loss of potency in the undiluted serum, and of increased potency upon dilution in the serum after the injection of the animal, but the effects on the whole are not striking.

TABLE XXX.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1801, injected with Crawford culture.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c.c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 50.	Diluted 1 to 500.	Diluted 1 to 5,000.	
F. 26	10 days after first injection	8,400	660	24,400	[a]∞	172,000	....
	9 days after second injection	5,900	4,160	19,800	∞	∞	∞
	14 days after third injection	2,400	60	[b]99,400	[b]196,000	[b]∞	∞
Crawford	10 days after first injection	840	0	640	7,000	1,460	....
	9 days after second injection	5,700	120	19,000	249,200	∞	∞
	14 days after third injection	13,000	6,220	[b]8,400	[b]12,500	[b]6,400	∞
G. P. 4692	10 days after first injection	2,060	3,500	2,540	3,820	2,120	....
	9 days after second injection	1,140	2,020	2,880	3,320	7,600	73,000
	14 days after third injection	7,300	7,800	[b]11,300	[b]8,100	[b]21,000	∞

[a] This character signifies that there were too many colonies to count.
[b] Diluted 1 to 10, 1 to 100, 1 to 1,000.

The hog (1801) in the experiment shown in Table XXX was injected on three different occasions with the Crawford organism. No tests were made of the serum before the inoculation of the animal, but there seems little or no evidence of the reaction usually seen in the serum of animals injected with bacteria in the tests which were made after each injection. The serum in most of the tests showed loss of bacteriolytic power on dilution, behaving in this respect like normal serum. It is true that with the homologous strain—Crawford—the serum ten days after the first injection and fourteen days after the third injection appeared to be somewhat more strongly bacteriolytic in the dilution of 1 to 5,000 and 1 to 1,000, respectively, than in the less diluted serum; but on the whole the serum seemed to act more like normal than like immune serum.

TABLE XXXI.—*Effect of dilution upon immune hog serum as compared with the effect of dilution upon the normal serum. Hog 1836, injected with culture F. 26.*

Culture used to test bacteriolysis.	Time blood was drawn relative to injection.	Number of bacteria introduced per 1 c. c.	Number of bacteria in serum after 24 hours.				Number of bacteria in check salt solution.
			Undiluted.	Diluted 1 to 10.	Diluted 1 to 100.	Diluted 1 to 1,000.	
Crawford	Before injection	32,200	[a]3,840	320	135,800	[b]∞	∞
	1 hour after injection	32,200	[a]5,340	240	∞	∞	∞
	3 days after injection	1,560	∞	0	151,400	25,200	42,000
F. 26	Before injection	30,400	[a]247,800	117,600	∞	∞	∞
	1 hour after injection	30,400	[a]∞	∞	∞	∞	∞
	3 days after injection	5,000	∞	98,000	∞	∞	∞
G. P. 4692	Before injection	35,000	[a]47,400	37,200	308,000	∞	∞
	1 hour after injection	35,000	[a]36,600	8,400	109,200	∞	∞
	3 days after injection	6,800	∞	13,800	75,600	285,600	553,600

[a] Diluted in the proportion of 1 part of serum to 3 of physiological salt solution.
[b] This character signifies that there were too many colonies to count.

In the experiment with the serum from the blood of hog 1836, Table XXXI, the animal was given intravenously 5 c.c. of a dilute beef-broth suspension of an agar culture of F. 26 strain. One day after the first injection the animal was given a second injection of the same amount of the same strain. The blood was drawn before any injection was made, one hour after the first injection, and three days after the second injection.

The results obtained with the serum from the blood of this hog, at least in so far as the strains G. P. 4692 and Crawford are concerned, are in striking contrast with the results obtained with the serum from the blood of hog 1801 (Table XXX). In the case of hog 1836 the animal was injected with a more virulent strain than was the case with hog 1836, and it is possible that this may have had some influence upon the results in the two cases. But aside from this the two sera must have had different potency originally, for even the normal serum of the blood of hog 1836, before the animal was injected, will be seen to have shown increase of potency upon dilution; the dilution of 1 to 10 was more potent than the dilution of 1 to 4. There was no test made of the undiluted normal serum for the reason that not enough of the blood was obtained to use for the purpose. This increase of potency on dilution of the normal serum of hog 1836 is to be seen particularly in the tests with the Crawford strain, in which the dilution of 1 to 4 showed 3,840 bacilli per 1 c. c. whereas the dilution of 1 to 10 showed only 320 bacilli after the same exposure—one day in each case—with the same number of organisms—32,200 per 1 c. c.—introduced. This serum in other words seems to have behaved somewhat like immune serum to start with, and this behavior seems to have been intensified by the injections, as will be seen by examining the table.

SUMMARY OF THE DILUTION EXPERIMENTS.

On summing up the results of the experiments made with normal and immune hog serum on dilution, it will be noticed that the normal serum seems to differ greatly in its behavior in different cases; that while it sometimes shows marked diminution of potency upon dilution it sometimes shows increased potency on dilution. The immune hog serum also shows variation in behavior.

But the results which would seem to be of especial interest are those in which the immune serum showed strong bacteriolytic power when undiluted, and lost in power on dilution in certain proportions, but gained potency upon further dilution. In these cases there appeared to be a combination of the behavior of normal and of immune serum, as this is usually stated to occur.

As already stated above, the characteristic behavior of immune serum is said to consist of the formation of bacteriolytic zones. A typical immune serum reaction consists of a pro-killing zone, a killing zone, and a post-killing zone on dilution. In the pro-killing zone, represented by the undiluted immune serum, there is no bacteriolysis. In the killing zone, represented by one or more dilutions, there is bacteriolysis. In the post-killing zone, represented by further dilution than the killing zone, there is again lack of bacteriolysis. But in the results at present under consideration the serum showed a killing zone to begin within the undiluted serum, thus imitating to this extent the normal serum.

The peculiar behavior just alluded to was to be seen in several instances given in the preceding tables. Perhaps the most striking examples are furnished by the behavior of the immune sera from hogs 1798 and 1808 (see Table XXIV). In these sera with the Crawford culture there was strong bacteriolysis shown in both cases undiluted. The 1808 was still strongly potent in the 1 to 50 dilution, but the 1798 serum was much less potent in this dilution than when undiluted. Both sera seem to have lost all potency when diluted in the proportion of 1 to 500. But the point to be specially noted is that they both seem to have gained greatly in the dilution of 1 to 5,000; particularly is this the case with the 1798 serum.

For the present it is merely necessary to call attention to these results; they will be taken up and discussed in another connection further along in the present paper.

GENERAL DISCUSSION.

Judging from the results of the above experiments as a whole, it seems apparent that the blood serum of hogs has but feeble bactericidal potency for *B. choleræ suis*, at least for the more strongly pathogenic strains. This would naturally lead to the presumption that the known susceptibility of hogs to infection by intravenous injection was due to this cause, but it does not seem as yet settled that the susceptibility of an animal in general is in direct proportion to the want of bactericidal power of its blood. In fact, in some instances, as is well known, quite the contrary has been shown to be the case. Nuttall, Buchner, and many others have shown that rabbit serum in test tubes will kill large numbers of anthrax bacilli, and yet a comparatively small number of these organisms introduced beneath the skin of a rabbit is certainly fatal to the animal. Guinea-pig serum, as recently shown by Buxton,[4] may be expected to kill approximately 1,000,000 typhoid bacilli per 1 c. c., and yet the injection of a relatively small amount of this organism intraperitoneally is certainly fatal for guinea pigs. In spite of this, however, as Wolff[20] has pointed out, it is probably all a matter of dosage, and bacteriolysis is an efficient means of defense where the number of bacteria concerned is not large. On the contrary, where the number of bacteria exceeds certain limits, bacteriolysis is not only not a means of defense, but it actually liberates the disease-producing substances—the endotoxins—from the bodies of the bacteria.

Pfeiffer[17] was the first to give this name, endotoxins, to the poisonous substances which are present in the bodies of the bacteria of certain species, and it is now quite generally the opinion that it is the liberation of these that brings about disease in infection with these bacteria. So long as bacteria of this kind remain intact, they are thought by those holding this view to be incapable of producing disease. Mechanical injury and other harmful effects formerly attributed to the action of bacteria are now thought by many not to exist.

The effects of inoculations of hogs with cultures of *B. choleræ suis*, and the conclusions to be drawn from these experiments are on the whole in harmony with those derived from experiments with various bacteria injected into rabbits, guinea pigs, and other animals. Thus Loeffler and Abel[12] found that undiluted immune blood serum from dogs failed to protect guinea pigs from injections of virulent colon bacilli. Neisser and Wechsberg[14] obtained analogous results with immune rabbit serum in test tubes, the undiluted immune serum failing to destroy the bacteria of the kind used to produce the immune serum. Buxton,[4] as already quoted, found that the undiluted serum of rabbits injected with cultures of typhoid or paratyphoid bacilli behaved in a similar manner. It was to be expected, therefore, that the serum of hogs injected with cultures of *B. choleræ suis* would show loss of bacteriolytic power in undiluted serum. The behavior of the diluted serum will be considered below.

In the foregoing experiments with the serum from the blood of hogs injected with cultures of *B. choleræ suis*, it appears that the effect of the first injection was sometimes, as stated, to deprive the undiluted serum of its power to kill *B. choleræ suis*. It is true that this behavior was frequently lacking. But if the results after several injections are examined, it will be found that the undiluted serum frequently regains its power. This is noticeable in a number of cases if not in all, and it perhaps accounts for the experience of so many observers that animals frequently die from injections of comparatively small doses after having been treated with carefully graded amounts of culture. Thus Wolff[20] found that strong, healthy rabbits, instead of becoming more and more accustomed to injections of cultures, withstood these less and less readily, even without increasing the dose; that after remaining apparently unaffected by previous injections, they often die suddenly on the inoculation being repeated with the same sized dose. This supersensitiveness on repeated injections has often been observed in various animals and with various bacteria. It has also been met with in the course of the present experiments with hogs. In fact, it does not seem so difficult to grade the first dose so as to avoid killing the animal as it is to strike upon the proper subsequent doses. The return of bacteriolytic properties in the serum following further injection of animals may account for this result, the first injections depriving the serum of the bacteriolytic power and thus preventing the liberation of endotoxin and its consequences, and the subsequent injections producing a return of bacteriolytic power and causing the liberation of the endotoxin.

It would seem perhaps difficult to reconcile the fact that the serum becomes more potent for the protection of animals other than the individual which produces it, and that yet from the very fact of its bacteriolytic power it should be injurious to the animal furnishing it. It would appear paradoxical to find that serum obtained from an animal possessed the property of protecting other animals, and, at the same time, not only failing to protect the animal furnishing it, but actually increasing the susceptibility of this animal. Here again Wolff offers the explanation that it is a matter of dosage. If the number of bacteria injected into the animal furnishing the serum, or into the animal which has been given a protective dose of the serum, is not so large that on being disintegrated the amount of toxin is sufficiently large to cause disease, the animal escapes. There are apparent difficulties in the way of this explanation, but it is, perhaps, nevertheless not without weight.

In this connection the results obtained by Elischer and Kentzler[6] on comparing the bactericidal power of unheated immune serum—i. e., immune serum containing its own proper complement—with the same serum heated and reactivated by the addition of fresh rabbit serum would seem to apply. The latter was found to be more potent than the former, and this would seem in a way to offer an explanation of the protective power of immune serum for an animal into which it is injected on the one hand and of the failure of such protective properties of the same serum for the animal from which it was obtained on the other. Elischer and Kentzler interpret their results as indicating that immune serum contains an insufficient amount of complement for the full development of its bactericidal properties. So in other cases, the amount of complement in immune serum may be insufficient to enable the serum to protect the animal from which it was obtained, while it would find sufficient complement in the serum of a fresh animal. To state this view briefly, the excess of amboceptors in immune serum finds the requisite amount of complement in the normal serum of uninfected animals.

When the behavior of hog serum on dilution is considered, the explanation afforded by the theory of complement diversion seems applicable, not only for the behavoir of immune serum in which there was shown often to be increased bactericidal potency on dilution, but also in the case of normal serum, as it will be attempted to show.

It will be recalled from the statements in regard to the theory of complement diversion given on page 13 that in accordance with the views of the Ehrlich school generally it is held by Neisser and Wechsberg, the originators of the theory, that bacteriolysis is due to the action of a substance called complement, which is present in normal and in immune serum, but that this substance can act on the bacteria only when it becomes united to these through the medium of certain bodies called amboceptors. The complements are held to be incapable of uniting directly with the bacteria. Now, according to the theory of complement diverson, if there are more amboceptors than complements present in any given serum, all the complements which are present unite with a corresponding number of amboceptors, and if any bacteria are introduced into the serum they unite with the free amboceptors in preference to uniting with those having complements attached. In this way the complements become diverted from the bacteria by the excess of amboceptors. It will also be recalled that while there are amboceptors in normal serum, these become increased when the animal from which the serum is obtained is injected with bacteria. The amboceptors formed by these injections are specific; that is to say, an animal injected with *B. choleræ suis* develops amboceptors in the serum which are capable of uniting with *B. choleræ suis* only. Reference to figure 4 (page 51) will serve to elucidate whatever may be obscure in the above explanation of the theory of complement diversion.

Bearing in mind that normal serum contains a relatively large amount of complement and a relatively small number of amboceptors, it is evident that by introducing normal serum into immune serum it is possible to increase the amount of complement without adding materially to the number amboceptors. In fact, by making a series of dilutions of immune serum and adding to each lot in the series a definite amount of normal serum, it is possible to obtain a set of dilutions containing various amounts of amboceptors and approximately the same amount of complement. Evidently if the immune serum is merely diluted and no normal serum is added, the complements present will be diluted in the same proportion as the amboceptors.

If the amount of complement is kept constant in the way above described, by the addition of a constant amount of normal serum, it is evident that in some of the dilutions of the immune serum the amboceptors will have enough or more than enough complements to combine with the amboceptors, and that in such cases the conditions necessary for bacteriolysis will be fulfilled—the amboceptors with the complements attached will unite with the bacteria. In other words, the undiluted immune serum, having an excess of amboceptors over complements, fails to show bacteriolysis, while the diluted immune serum with complements added, having no excess of amboceptors over complements, causes bacteriolysis.

Buxton[4] has raised the objection to the theory that it can account for those cases only in which the amount of complement is kept constant in the dilutions of immune serum, while the amboceptors alone are diminished. He makes the point that in experiments such as are recorded in the present paper, in which amboceptors and complements are diluted to an equal extent, the theory of complement diversion is inapplicable; and since the process is probably the same in both cases, both where the complement is kept to a constant amount by heating the serum and adding a definite quantity of normal serum on the one hand and where the serum is not heated and has no normal serum added on the other hand, the theory as a whole is not tenable. Valid as this objection appears from Buxton's presentation, it appears on further consideration as scarcely to be insuperable, for the theory seems to afford a satisfactory explanation in every case where the amboceptors are in excess of the complements, and, granting a certain proportion between these in the undiluted serum, it is not necessary for the complement to be present in an equal amount in the undiluted serum and in the dilutions for the occurrence of the diversion of the complement to take place. All that the theory would seem to demand is that the undiluted serum contain an insufficient amount of complement to satisfy the amboceptors which are free and those which are attached to the bacteria. Bacteriolysis will take place just in proportion to the paucity of the free amboceptors, for, according to the theory, those bacteria which are not coupled to free amboceptors are liable to bacteriolysis by becoming attached to the amboceptor-complement combinations.

In figure 4 it is attempted to show that complement diversion may well account for those cases in which the complement is diluted in the same proportion as the amboceptors. This point could be more strikingly shown if a larger number of amboceptors and complements could be taken, and a much greater relative difference could be made in the diagram between the number of amboceptors and of complements, but for obvious reasons it is impracticable to do this. Thus, if it were feasible to show 100 amboceptors and 100,000 amboceptor-complement combinations in 1 c. c. of undiluted serum, it could be demonstrated that 100 bacteria introduced would all escape bacteriolysis in this serum, only 10 bacteria would escape in 1 c. c. of a dilution of 1 to 10 of the same serum, only 1 bacterium would escape in 1 c. c. of a dilution of 1 to 100, and none would escape in 1 c. c. of a dilution of 1 to 1,000. In other words, theoretically in serum in which the above conditions prevailed, taking 1 c. c. in every case and introducing 100 bacteria, there would be no bacteriolysis in the undiluted serum, but complete bacteriolysis in the same serum in the 1 to 1,000 dilution.

In figure 4 the amboceptors are represented by the parts marked *a*, the complements by the parts marked *k*, and the bacteria by the parts marked *b*. No. 1 is intended to represent the undiluted serum, and all except two of the amboceptors are shown as having diverted a corresponding number of complements, while the two amboceptors, which are not united to complements, and which represent the excess of these bodies over complements, are united to a corresponding number of bacteria. Evidently in such a case there would be no bacteriolysis, since all the complements have been diverted from the bacteria. In No. 2 half the amount of the serum, as in the first case—that is to say, 1 c. c. of a 1 to 1 dilution—is represented, with the same number of bacteria added as in the undiluted serum represented by No. 1. Under the conditions represented by No. 2 it is evident that one-half of the bacteria would be killed. In No. 3 is represented 1 c. c. of a dilution in which 0.25 c. c. of the original serum is contained. In this case all the bacteria would be killed, since there are no longer any free amboceptors to unite with the bacteria, or, at any rate, there would not be enough of the amboceptors to divert the complement in all of the four portions into which it is assumed the serum is divided. If the amboceptors are to be regarded as chemical substances in solution, then there would not be enough in any one of the four portions to cause diversion of the complement. If, on the other hand, the

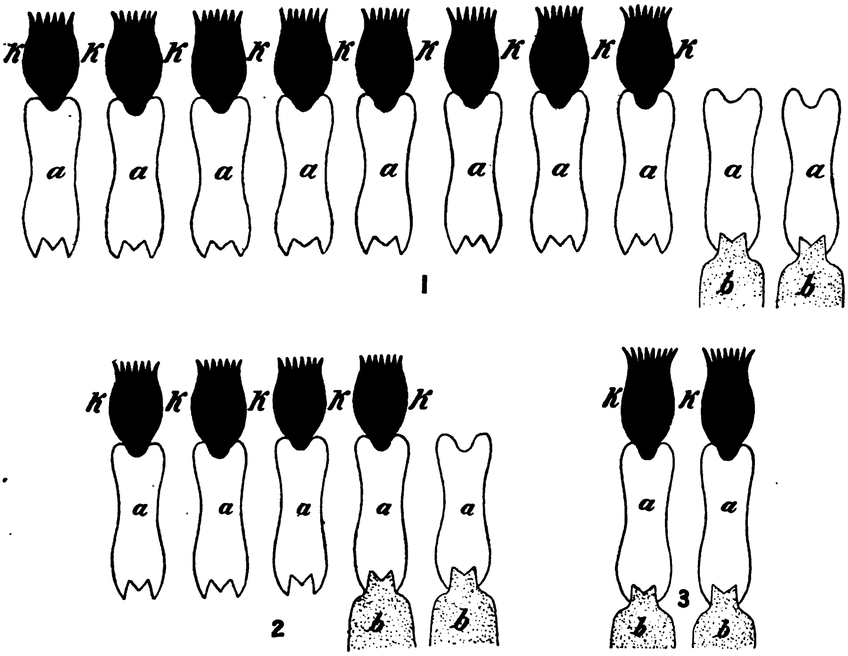

FIG. 4.—Diversion of complement in immune serum, not heated, and without the addition of foreign complement.

amboceptors are bodies in the strict sense, then it is evident that two of the four portions would show bacteriolysis of one-half of the bacteria, while the other two would show complete bacteriolysis, assuming uniform distribution of the amboceptors. In either case the diluted serum would be more powerfully bactericidal than the undiluted serum. It will be observed that the complement is not kept at a constant amount in the dilutions represented by the diagrams, but that it is diluted simultaneously and to a like degree with the amboceptors.

From what has been said above it would appear that diversion of complement seems a reasonable explanation of the phenomena which are observed in unheated unreactivated serum, and it therefore seems to overcome Buxton's objection to the application of the theory to the phenomena observed under such conditions.

It is aside from the purpose of the present article to try to meet in general objections to the theory of complement diversion, or to discuss this theory apart from whatever bearing it may have upon the present experiments; but incidentally the objection raised by Buxton of bringing in accord with the theory of complement diversion the fact that Asiatic-cholera spirilla are killed about as readily by typhoid and paratyphoid immune serum as by normal serum may perhaps be overcome by consideration of the fact that the injection of an animal with any one kind of bacterium probably has no effect upon the amboceptor-complement combinations for any other kinds of bacteria which are present in the serum of the animal undergoing inoculation. The effect is probably very sharply specific, even going so far in some cases as to affect different strains of the same organism, as is shown by the results of some of the experiments in the present paper. It seems not at all inconceivable, at least, that an animal injected with any given kind of bacterium might well be deprived of the power of killing the kind of bacterium with which it was injected, owing to the excessive production of specific amboceptors, without interfering with the amboceptor-complement combinations for any other bacteria which may be originally present.

In view of the very specific reaction which takes place on injecting an animal with bacterial cultures, there would really seem to be no good reason why immune serum should act differently from normal serum for any but the homologous organism. The amboceptors which are newly formed in consequence of the injections could not reasonably be supposed to have the power of depriving the amboceptors originally present of the complements to which they are attached. If it is true, as assumed, that in normal serum there are amboceptor-complement combinations which will destroy *B. choleræ suis*, and in the same serum amboceptor-complement combinations which destroy *B. typhosus*, the injection of the animal with cultures of *B. choleræ suis* would evidently increase the amboceptors for this organism, and these newly formed amboceptors would have the power of uniting with any free complement, but not with any complement already united to the *B. typhosus* amboceptors. So that while the newly formed amboceptors would and probably do find free complements with which to unite, they could hardly be expected to tear loose the bonds between the original amboceptors and complements.

As has been pointed out by Neisser and Wechsberg in their experiments, the increase of bacteriolysis usually observed on the dilution of immune serum does not appear to be explicable by the conception of Bordet that the immune body—the amboceptor—is merely a sensitizing agent. For if the amboceptor were in reality merely a "substance sensibilisatrice" it is not likely that immune serum would be more active when dilute than when concentrated. At least it would be only reasonable to presume that a reagent possessing the properties ascribed to the sensibilisatrice by Bordet and his followers would lose rather than gain in power upon dilution, and that a given amount of the undiluted sensibilisatrice would be more potent than the same amount of the diluted substance, as is the case with other reagents. On the other hand, Ehrlich's conception of the amboceptor affords a satisfactory explanation of the behavior of immune serum upon dilution, as has been shown in the above discussion of the theory of complement diversion.

The results obtained in the present investigation in which the hog serum appeared to increase in potency upon dilution seem best explained by the theory of complement diversion. But in these experiments, as in all others of like nature, it would seem possible that the process of bacteriolysis or the failure of bacteriolysis may at least be complicated by the formation of antibacteriolysins on the injection of the hogs or by the presence of antilysins normally present in the serum. Opinions are divided upon the question as to whether there are such antilysins present in normal or in immune serum. But if there should be such antibodies present, it is reasonable to suppose that these would be weakened in power on diluting the serum, and that consequently such serum would be more potent when diluted than when undiluted, unless the lysins and antilysins should be diluted in like proportions.

There seems to be no difference of opinion in regard to the development of qualities antagonistic to bacteriolysis in serum under certain circumstances, but the matter in dispute is whether this antagonistic property is due to special antilysins or whether it is due to the presence of nonspecific, normal amboceptors or of superabundance of immune amboceptors which absorb the complement, and in this way prevent bacteriolysis.

Pfeiffer and Friedberger,[16] who were the first to detect this antago-

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nistic action on the part of normal serum, hold that under certain circumstances specific antibodies are formed, which combine with the complement. In other words, they regard the antilysins as anticomplements. Their experiments consist of triturating normal serum with bacteria and centrifugalizing. Under such circumstances the supernatant serum acquires the property of neutralizing the bacteriolytic power of immune serum. Thus normal serum, which in itself exerts no antibacteriolytic power, becomes antibacteriolytic for cholera immune serum when it is triturated with cholera bacilli and centrifugalized or filtered through a bacteria-proof filter.

Gay[10] attributes antilytic properties developed in this way to the formation of specific precipitines. He finds that only when there is a specific precipitation is there any antilytic property shown by hemolytic sera, and since hemolysis and bacteriolysis are probably identical processes, the same statement would apply to the latter as well as to the former.

Gay's view has been called into question by Sachs,[18] who attributes the antilytic properties of serum to normal, nonspecific amboceptors which he regards as acting as neutralizers of the complement. The only difference between the explanation offered by Pfeiffer and Friedberger on the one hand and Sachs on the other is that while the former look upon the antilysins formed in their experiments as newly formed bodies derived in part from the bacteria, Sachs contends that the antagonistic bodies are present in the serum from the start. He states, at least, that this is the only point in which he takes issue with Pfeiffer and Friedberger on the matter of the formation of antilysins for hemolytic serum.

Lipstein,[11] on comparing strongly agglutinating immune sera, found that while both the specimens examined possessed equal agglutinating power only one of them showed the phenomenon of complement diversion—that is to say, antibacteriolytic properties. He was also unable to discover any constituent of the normal serum which showed diversion of complement, but on the contrary he found this phenomenon to take place in immune serum only. In other words, the antagonistic action of serum to bacteriolysis he attributes solely to the diversion of complement by the specific, immune amboceptors.

In spite of this conflict of authority, it would seem at least that the Neisser-Wechsberg phenomenon permits of easiest explanation by the theory of complement diversion. For if the failure of the undiluted immune serum to produce bacteriolysis were due to the presence of antilysins, it would be less potent upon diluting the serum, it is true, and consequently exert less power upon the bacteriolysins when diluted than when undiluted, but the relative effect would remain the same. It is nevertheless probable that if bacteriolysins are present in immune serum they complicate the process.

While the theory of complement diversion seems to have been used by all investigators to explain the bacteriolytic phenomena of immune sera only, it would not seem inapplicable to those which take place in normal serum as well. The theory seems at least to explain the behavior of the normal serum of hogs upon dilution in the present investigation. Even in those cases where the normal serum showed diminution of potency on dilution, the diminution was not in direct proportion to the dilution, as has been previously noted; but on the contrary the diluted normal serum acts more powerfully, relatively, than the undiluted normal serum. This relatively greater potency of the diluted normal serum over the undiluted may be accounted for by the theory of complement diversion, if it is assumed that there is an excess of amboceptors over complements in the normal serum of hogs to start with, as certain considerations will show.

Difficulties in the way of the acceptance of the explanation just offered are of course very obvious. In the first place, normal serum generally has been found to contain an excess of complement over amboceptors; in fact, in adding normal serum to heated immune serum, this is done for the purpose of increasing the amount of complement without adding to the amboceptors. In heating immune serum it is the complement which is rendered for the time inactive, as it is called, and the addition of fresh serum reactivates the immune serum by means of the new complement contained. The addition of fresh serum to the heated typhoid immune serum of a rabbit reactivates the immune serum. On account of the large amount, or of the great number, of complements contained in the serum from the blood of a normal horse this can be used to reactivate several different kinds of immune sera. Then again, as stated above, in the process of immunization, the newly formed amboceptors probably find free complements with which to unite in the serum of the animal undergoing injection.

While the force of these and of possibly other objections can not be denied, it would seem evident from the experiments herein reported that the only difference between the behavior of immune serum on the one hand and of normal serum on the other, upon dilution, was a difference of degree and not of kind. If this is true, the theory of complement diversion must be applicable to both or to neither.

Evidently there are only three possibilities in regard to the relative number of amboceptors and of complements in any given serum—either there is just the proper proportion of amboceptors to complements or there is an excess of the one or of the other of these. If the amboceptors and complements are present in just the proper proportion, then there would be a simple relation between the undiluted and the diluted serum. The serum would become relatively and actually less and less potent in exact ratio to the extent of the dilution. If a given number of bacteria were killed by a certain amount of the undiluted serum, a certain fraction of this number would be destroyed by the same fraction of the serum. If the complement is in excess, evidently the same rule would hold; the free complements would neither help nor hinder the process. But if there were an excess of amboceptors, a part of the bacteria corresponding to the amount of this excess would escape bacteriolysis, according to the theory of complement diversion. On dilution, however, there would be relatively more bacteria killed where there are free amboceptors present in the undiluted serum, as already explained in the description of the Neisser-Wechsberg theory.

Since none of the experiments in the present investigation have been made to determine whether the serum from normal hog's blood contains free amboceptors, and no experiments of the kind with hog's blood seem to have been so far reported, the matter must for the present be left undecided. But the assumption of such a condition in the normal serum would seem necessary to account for the results obtained.

A specific example taken from one of the experiments already described may serve to show the application of the theory of complement diversion to the phenomena observed on diluting normal hog sera in so far as the effect of dilution upon the bacteriolytic potency is concerned. Into the undiluted serum from the blood of hog 1859, and into each of the various dilutions, there were introduced before injection of the animal 3,760 bacilli of the Crawford strain per 1 c. c. of the serum. The number of bacilli surviving after twenty-four hours in the undiluted serum was 80 per 1 c. c. There are two conditions which may be assumed to exist in the serum, either of which would account for the escape of the 80 bacilli, disregarding for the present the fact elsewhere discussed that there is a multiplication of the bacteria surviving bacteriolysis and assuming that the 80 bacteria in the present case represent merely those bacteria which escaped bacteriolysis. Either there were not enough amboceptor-complements for all the bacteria introduced—in other words, there were 80 short of these—or, if there were enough or more than enough amboceptor-complements, there were also 80 free amboceptors. Either of these two conditions would account for the escape of the 80 bacteria in the experiment. A simple calculation from the result obtained on diluting the serum will show which of these conditions is most likely to have prevailed. If there had been a paucity of amboceptor-complements—if there had been, in other words, just 3,680 of these instead of over 3,760, which was the number of bacteria added—then a dilution of 1 part of the serum to 9 parts of salt solution should have killed only 368 bacilli per 1 c. c. when these were introduced; whereas, as a matter of fact, this dilution killed 2,600 per 1 c. c. There would appear, therefore, no ground for assuming the failure of bacteriolysis in the immune serum to have been due to a paucity of amboceptor-complements in the undiluted serum.

Suppose, now, that the other condition mentioned above existed in the serum, namely, that there was an excess of amboceptors over and above the amboceptor-complements, and that there were more amboceptor-complements than the bacteria introduced. A simple calculation will show that such a supposition will readily account for the results obtained in the diluted serum as compared with the results in the undiluted. If there were in the undiluted serum 26,000 amboceptor-complements and 80 free amboceptors per 1 c. c. then on dilution of 1 to 10 there would evidently be 2,600 amboceptor-complements and 8 free amboceptors per 1 c. c. of the diluted serum. Consequently 8 bacteria would escape bacteriolysis on account of being attached to the free amboceptors, but 2,600 would be destroyed.

The above calculations are, of course, not intended to indicate precisely the processes involved, for these are probably complicated by the multiplication of the surviving bacteria, as mentioned above. Still, even if such a complication is admitted, the probabilities in favor of the condition last named become even greater, as a moment's consideration will show.

A further complication may also arise from the possibility, however remote, that the amboceptors and complements are not uniformly distributed in the serum—that while they may perhaps usually be so distributed, they may be found more abundantly in one part of the serum than in another.

SUMMARY OF THE CONDITIONS AFFECTING BACTERIOLYSIS.

The conditions under which bacteriolysis would take place, and those under which no bacteriolysis would take place in any given serum, may be summarized as follows:

1. Complete bacteriolysis could take place only where there were no free amboceptors and where there were at the same time a number of amboceptor-complements equal to or greater than the number of bacteria introduced.

On dilution in a serum of this kind there would be a loss of bacteriolytic power in proportion to the degree of dilution if the amboceptor-complements were exactly equal in number to the bacteria introduced. If there were more amboceptor-complements originally in the undiluted serum than the bacteria introduced, then on dilution there would be relatively more bacteria destroyed. If the excess of amboceptor-complements is large enough, there could of course be enough present in the diluted serum to kill as many bacteria as were killed by the serum before dilution.

2. Partial bacteriolysis would follow when there were fewer amboceptors present in the serum than the bacteria introduced and when at the same time there were any amboceptor-complements present.

The extent of bacteriolysis upon dilution would depend upon the number of amboceptor-complements present originally.

3. No bacteriolysis could take place if the free amboceptors were equal in number to the bacteria introduced, or if they were in excess of this number, either in the undiluted or the diluted serum.

THE THEORY OF COMPLEMENT DIVERSION.

In spite of the serious difficulties alluded to it would nevertheless appear that the theory of complement diversion gives an insight into the process of bacteriolysis occuring in normal serum in so far as this is affected by dilution—in those cases at least where the experiments show that the serum is actually or relatively more potent upon dilution than when undiluted—irrespective of the nature of the serum, whether this is normal or immune. But this would leave the results obtained in certain other experiments still unexplained. It will be recalled that in certain experiments the serum was found to be bactericidal when undiluted, that it lost in bactericidal potency on dilution, but that it gained in potency upon further dilution. It is these results which would be left unexplained by the theory of complement diversion as thus far discussed. One example of this behavior among others is to be seen in Table XXVI, in the serum of hog 1798, ten days after the inoculation of the animal with the F. 26 strain of *B. choleræ suis*. In one of the tests there were introduced into the undiluted serum and into the various dilutions in round numbers 800 Crawford bacilli. The result after twenty-four hours was that all the bacilli were destroyed in the undiluted serum, while in the dilutions of 1 to 50 and 1 to 500 there was an increase of about thirty-three fold and one hundredfold, respectively. But in the dilution of 1 to 5,000 there were only 140 bacilli remaining. In other words, the undiluted serum showed strongly bactericidal power, and the dilution of 1 to 5,000 also showed considerable potency, while the dilutions between these two showed at most but feeble power. If this result is analyzed, it will be seen that the serum appeared to act like normal serum at the start when undiluted and in the first dilutions, but that on further dilution it appeared to act like immune serum. This suggests that possibly the normal amboceptor-complements persisting in the serum may have been more potent than the immune amboceptors formed in consequence of the injection, that the first effect of dilution was to reduce the potency of the normal amboceptor-complements, and that further dilution enabled the immune amboceptors to produce the phenomenon of complement diversion. This would be quite in accord with certain views expressed elsewhere in this paper to the effect that the injections of cultures or their products while they cause the formation of new amboceptors leave the normal amboceptor-complements unaffected.

In the above discussion it is assumed that the two bodies concerned—the amboceptor and the complement of Ehrlich, the sensibilisatrice and the alexin of Bordet—are capable of uniting and do actually unite independently of the presence of bacteria or of other cells. But Bordet[3] has recently published a series of investigations tending to show that the experiments of Ehrlich and Sachs[5], which constitute the chief evidence in favor of this view, are capable of quite a different interpretation from this, and that this interpretation is in fact not justifiable from the results of the experiments which consisted in the demonstration of the fact, not denied by Bordet, that ox serum will produce cytolysis only when the serum has in it amboceptors and complements simultaneously. It is not possible, as in some other cases, to produce cytolysis by sensitizing cells with ox amboceptors—that is, with heated ox serum—and, after washing these sensitized cells, adding complement—that is, fresh serum. Cytolysis with ox serum takes place only when the heated ox serum and some unheated fresh serum (horse serum was the kind used in the experiments) are employed at the same time. This is interpreted by Ehrlich and Sachs as showing that while the free amboceptors present in the ox serum will not unite with the cells they will and do so unite when they are previously attached to complements. But Bordet's results appear to show quite plainly that in this case the horse serum which was used as complement produces cytolysis quite independently of the ox serum, and that while cytolysis takes place more promptly when heated ox serum—ox amboceptors—are added, the ox serum is not necessary. Bordet therefore regards the experiments as showing that the heated ox serum acted merely as an accelerator of cytolysis.

Bordet summarizes his conclusions as follows:

> We see but one rational explanation of the peculiar action of ox serum—that there exists in the serum a peculiar substance capable of resisting heat of 56° C. and which remains unaltered for many months in this heated serum. The substance is probably of an albuminous or colloid character, and does not adhere to the normal corpuscles, but is precipitated upon the corpuscles which are previously charged with sensibilisatrice and alexin. We believe that it is a veritable process of glueing of absorption depending upon molecular adhesion. * * * In conformity with the statements of Ehrlich and Sachs, experiments show that the corpuscles of guinea pigs become hemolyzed in a mixture of fresh horse serum and of ox serum, the latter having been heated to 56° C., while they resist hemolysis if they are first subjected to the action of the heated ox serum and have the horse serum added subsequently. But the interpretation offered by Ehrlich and Sachs, according to which the sensibilisatrice furnished by the ox serum does not unite with the corpuscles unless it (the sensibilisatrice) is previously connected with alexin derived from the horse serum is not correct. In the first place, the sensibilisatrice, which plays a preponderating and most essential rôle, is not contained in the ox

serum at all, but is furnished by the horse serum. Consequently these sensibilisatrices behave like all of their congeners, in the sense that they do not require the presence of the alexin before they are capable of uniting with the corpuscles.

Finally, this interpretation leaves completely in the dark the very special peculiarities of the cases of hemolysis in question.

The peculiarity of ox serum consists in the presence of a certain element which resists 56° C., and also resists standing, and is of the nature of a colloid, doubtless albuminoid, and which, furthermore, is absorbed by corpuscles which have become charged with sensibilisatrice and alexin, but which remains free in the presence of normal corpuscles or of corpuscles merely sensitized—i. e., corpuscles treated with heated serum alone. The absorption of this colloid by corpuscles which have been treated with both sensibilisatrice and alexin has the effect of energetically agglutinating them and of rendering them more susceptible to hemolysis except under certain circumstances. * * * The absorption of the sensitized and alexinized corpuscles is very likely due to molecular adhesion, the preliminary treatment having modified the corpuscles in so far as their adhesive properties are concerned. Under these conditions the absorption may take place independently of the species of animal from which the corpuscles are obtained; it may even take place with the corpuscles of the same animal which furnishes the colloid, as in the case of ox serum.

Now, if the contention of Bordet as set forth above is correct, and if the two bodies concerned in cytolysis do not unite, then of course the theory of complement diversion must fall, since it requires as the first condition the union of amboceptors with complements. Nevertheless, there appears to be as yet no other explanation of the phenomena observed on diluting immune serum, and, as an attempt has been made to show in the present paper, there seems to be no other explanation of the behavior of normal serum on dilution.

CONCLUSIONS.

In summarizing the results of the present investigations it is seen—

1. That the bactericidal potency of the serum from the same hog varies from time to time.
2. That the serum from one and the same drawing differs in potency for different strains of *B. choleræ suis.*
3. That while the effect of standing is to weaken the potency, this effect is more marked in some specimens of serum than in others under the same conditions.
4. That the bactericidal power of the serum from the venous blood of hogs is not always more potent than that from the arterial blood.
5. That heating the serum at about 54° C. for thirty minutes inactivates the serum.
6. That the Neisser-Wechsberg phenomenon is sometimes seen in and sometimes missed from the blood of hogs injected with cultures of *B. choleræ suis.*
7. That the theory of complement diversion may not only account for the behavior of immune serum, but also for that of normal serum on dilution, or, at least, that the theory, if applicable in the one case, is equally so in the other.

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BUREAU OF ANIMAL INDUSTRY.—BULLETIN No. 96.

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JUL 19 1907

A COMPARATIVE STUDY OF TUBERCLE BACILLI FROM VARIED SOURCES.

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., January 8, 1907.

SIR: I have the honor to transmit herewith a paper entitled "A Comparative Study of Tubercle Bacilli from Varied Sources," by Drs. John R. Mohler and Henry J. Washburn, of the Pathological Division of this Bureau, and to recommend that the same be published as Bulletin No. 96 in the series of this Bureau.

This work comprises a series of experiments upon several species of animals with the main object of shedding further light upon the question of the intertransmissibility of the human and bovine types of tubercle bacilli. The investigation has brought out what is regarded by the authors as strong additional evidence for the affirmative. It is unnecessary, therefore, to point out its bearing upon the fight which is being waged against tuberculosis and the importance of excluding from human consumption the meat and milk of tuberculous animals.

Respectfully,

A. D. MELVIN,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

CONTENTS.

Page.

Introduction — 7

Object of the investigation — 8

History of similar investigations — 9

Origin of cultures used in present investigation — 20

- The cultures selected for the tests — 25

Technique of the experiments — 26

- Isolation of cultures — 26
- Preparation of suspensions for inoculations — 27
- Dosage — 28
- Staining of bacilli — 29

Appearance of cultures on dog serum — 29

- Characteristics in subcultures — 33

Morphological examinations — 33

- Review of morphological results — 36

Pathogenesis — 38

- Virulence for rabbits — 38
  - Results of rabbit inoculations — 39
  - Summary of rabbit inoculations — 44
- Virulence for white mice — 46
- Virulence for cats — 47
  - Results of cat inoculations — 47
  - Summary of cat inoculations — 50
- Virulence for dogs — 53
  - Results of dog inoculations — 54
  - Summary of dog inoculations — 56
- Virulence for goats — 58
  - Results of goat inoculations — 58
  - Summary of goat inoculations — 60
- Virulence for sheep — 60
  - Results of sheep inoculations — 61
  - Summary of sheep inoculations — 63
- Virulence for cattle — 64
  - Results of cattle inoculations — 65
  - Summary of cattle inoculations — 73

Histologic examination of lesions produced — 74

Chemical reaction of glycerin-bouillon cultures — 78

Conclusions — 82

Bibliography — 85

ILLUSTRATIONS.

		Page.
PLATE I.	Various forms of human tubercle bacilli	32
II.	Various forms of human and bovine tubercle bacilli	32
III.	Various forms of bovine tubercle bacilli	32
IV.	Lung of sheep No. 104, inoculated subcutaneously with human tuberculosis (Boy V)	64
V.	Lung of heifer No. 302, inoculated subcutaneously with human tuberculosis (Boy V)	64
VI.	Miliary tuberculosis affecting the lung of heifer No. 297, inoculated subcutaneously with human tuberculosis (BB)	64

A COMPARATIVE STUDY OF TUBERCLE BACILLI FROM VARIED SOURCES.

INTRODUCTION.

For many years tuberculosis has been regarded as one of the most serious affections common to man and animals. Because of the similarity of the microscopic lesions produced by this disease in all species of the domestic animals, as well as in man, it was formerly considered that, wherever found, the affection was sufficiently described by the simple name of tuberculosis. With the announcement of the discovery of the specific micro-organism of tuberculosis by Robert Koch, in 1882, there was awakened a new interest in the study of the disease, and as a result of this (from a bacteriologic view point) classic investigation of the affection in its various phases, it was accepted on all sides as a fact that tuberculosis, whether found in man, bovine, or bird, owed its origin in every instance to one and the same species of bacillus. Aided soon afterwards by Ehrlich's discovery of the peculiar staining characteristics of the organism, nothing to prove the identity of the slender bacillus obtained from tubercular lesions in various species of animals seemed lacking.

In 1896 Theobald Smith [30][a] called attention to the existence of certain differences between tubercle bacilli obtained from cattle and those presumably from man, which he confirmed in 1898, but the subject failed to arouse any universal comment until Koch, in 1901, after repeating in substance the statement made by Smith, continued by expressing the opinion that there was but little danger of man ever becoming infected with tubercle bacilli of bovine origin, because of the fact that bacilli of human and of bovine sources were different and were not transmissible between human and bovine subjects.

The amount of research work which has since been performed by investigators in their attempts to answer some of the numerous questions which at once arose upon the admission of material differences between tubercle bacilli of varied origin is truly amazing. Medical literature has been flooded with expressions of opinion pro and con, and with reports of experiments made for the purpose of gaining further light on a vexatious question. Many valuable deductions have been obtained and much that is instructive has been achieved through these efforts.

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[a] The figure references refer to bibliography at end of bulletin.

OBJECT OF THE INVESTIGATION.

The work of which this article is a record was undertaken for the purpose of repeating the experiments performed by Smith, and of confirming or disproving the subsequent investigations of Koch, who, while in a general way verifying the results obtained by Smith, went much further in drawing his conclusions, asserting not only that there is a distinct difference between human and bovine tubercle bacilli, but that cattle can not be infected with the human bacillus and that man is not susceptible to the bovine organism, except on the rarest occasions. While Smith's conclusions were based upon an elaborate, well planned, and scientifically executed research, which included not only a full description of the morphology and cultural characterictics of the human and the bovine tubercle bacillus, but also parallel inoculations of five species of animals, Koch's assertions were apparently the result of a comparison of only the pathogenic effects of these bacilli on two species of animals.

The demands made by Smith for the repetition by other investigators of the work performed by him can be met, but the demands first made by Koch are only practicable in so far as they relate to the inoculation of cattle with bacilli of human origin, since direct proof that would be forthcoming by inoculating man with the bovine bacillus can not be obtained. And the later address by Koch in 1902 also postulates conditions incapable of proof. It has therefore been our endeavor to follow out as closely as possible the earlier investigations of Smith, which include the only practical experiments demanded by Koch—the inoculation of cattle with bacilli from man—stated by the latter to be the only test necessary to discriminate between human and bovine bacilli.

The practical importance of having exact knowledge of the intertransmissibility of human and bovine tuberculosis has long been realized by the sanitarian and was immediately recognized by the public when it became acquainted with the statements made by Koch. As a result of Koch's opinions health officers in the enforcement of their regulations at once met with opposition from the people who are always willing to grasp at a new thought or idea. But the results obtained by the majority of those who immediately undertook to solve this problem were in opposition to Koch, and they almost unanimously recommended that it would be unsafe to relinquish or modify the present sanitary police regulations with reference to bovine tuberculosis.

While our work has been carried out along the above-mentioned lines, other methods of experimentation suggested themselves and were inaugurated, but can not find a place in this paper. The objections that have been frequently raised to results obtained with old cultures, also by the injection of large doses and by certain methods of inoculation, have been met by working in all cases with freshly isolated bacilli from natural cases, by giving small, uniform, and well-proportioned doses, and by using the subcutaneous method of inoculation in all cases except in a number of cats, which received intrathoracic injections.

HISTORY OF SIMILAR INVESTIGATIONS.

As early as 1896 Theobald Smith[30] called attention to certain differences in morphology, biology, and pathogenesis which he had found to exist between bovine tubercle bacilli and a culture presumably of human origin. This was followed in 1898 by a more extensive work,[31] which later became world renowned because of the freshly discovered facts which it brought forward. It was here shown that tubercle bacilli of the bovine type are short, straight, not very regular in outline, grow less vigorously upon artificial media than sputum bacilli, are much less influenced by certain modifications of the culture medium, and, finally, tend to remain short when grown artificially through several generations, while the sputum bacilli grow profusely and quickly become long and slender during cultivation, if not so at the start.

A marked difference in pathogenic power was also shown between tubercle bacilli of the bovine type and those from sputum. In this experiment of Smith's the cultures of human tubercle bacilli were all obtained from sputum and no attention was given to cultures from tuberculous tissues.

Despite the merit and vast importance of this subject, very little attention was given these results by sanitarians until the appearance of the very radical statements of Koch in 1901. He, in collaboration with Schütz,[15] reached the conclusion, after a series of comparative tests by means of subcutaneous, intravenous, and intraperitoneal inoculations, as well as by feeding and inhalation tests, that cattle are not susceptible to bacilli obtained from human tuberculosis, while bacilli of bovine source invariably attack them with great rapidity and virulence. Reasoning from these discoveries, they advanced the opinion that bovine and human tuberculosis were sufficiently different to preclude their intertransmissibility from one of these hosts to the other. They also suggested that one could readily determine by inoculating certain tubercle bacilli beneath the skin of cattle whether they were derived from a human or a bovine subject. Immediately following these statements, it was very natural that numerous investigators should direct their energies toward the settlement of the contested point—that members of the human family were not susceptible to bacilli of bovine origin, and, conversely, that bovidæ could not be successfully inoculated with tubercle bacilli from a human source. Since human and bovine bacilli comprise the most important types known, much of the comparative work has been centered around them in previous work, and they will also form the basis of the present article. In the five intervening years many instances have been cited of the infection of man through cuts and injuries received while slaughtering tubercular cattle. Numerous attempts have been made to infect cattle through various methods of inoculation with human sputum, or with cultures derived from human cadavers. The results have been varied, but the conclusion of the majority of the investigators is strikingly uniform.

Even at the time that Koch and Schütz were making their comparative investigations Ravenel[27,28] was actively employed in similar experiments. His conclusions were very different from those of Koch and Schütz, as will be seen from the following extracts from an address in which his work and its deductions were clearly described:

> While it has been shown conclusively that a greater pathogenic power is the most distinguishing characteristic between the bovine and human tubercle bacilli, even this is not constant.
>
> The pathogenic action of the bovine tubercle bacilli for man is seen where a careful study of wound infection is made. A comparison with similar infections with human tuberculous material shows that the bovine bacillus grows well in the human body, under the most unfavorable circumstances producing typical lesions, and that it shows for man at least as great pathogenic power as the human bacillus under identical circumstances. * * * The most direct and positive proof that bovine tuberculosis is responsible for a certain amount of human tuberculosis is given by the finding of the bovine bacillus in lesions of man. In June, 1901, I succeeded in isolating from the mesenteric glands of a child sent to me by Dr. Alfred Hand from the Children's Hospital, in Philadelphia, a culture which has been designated BB. This culture showed the most intense virulence for cattle, killing two calves in seventeen and twenty-seven days, respectively, and a 6-year-old cow in seventeen days. Excepting in the first generation, when some irregularity of staining was noticed, this culture has always shown every characteristic of the bovine tubercle bacillus, and must be regarded as having come directly from cattle. * * * I have since isolated two other cultures from mesenteric glands of children which have unusual virulence.

Culture U produced general infection in a calf with moribund condition in forty-six days, and the other possessed even greater virulence for cattle and was considered bovine by Ravenel.

Vagedes (1898)[34] was one of the earliest writers to notice differences in virulence and morphology between various cultures of tubercle bacilli, but his investigations did not serve to place human and bovine tubercle bacilli in separate classes, as he found certain human cultures which he rated with his bovine cultures in view of their virulence for test animals and in view of their shortness of form when grown artificially.

A test of the comparative virulence of eight cultures of tubercle bacilli which were all derived from human sources was made by Veszprémi (1903)[35], with the result that the greatest variation in the degree of virulence was determined. The tests of Veszprémi were based upon the inoculation of rabbits, and he found that no constant standard of virulence can be established for tubercle bacilli from human sources. The avirulent cultures disappeared from the rabbit's organism without leaving behind any trace. Those of slightly greater virulence, when used in sufficiently large quantities, possessed the power of producing tubercles, but no caseation developed, and there appeared to be no increase in the number of tubercle bacilli within the tissues. When tubercle bacilli of a still higher degree of virulence were used, the lesions produced by them were very similar to those caused by the use of tubercle bacilli of slightly lower virulence, but they retained the capability of multiplying within the tissues and probably also of generating a slightly toxic agent. The most virulent cultures produced tubercles in which the tubercle bacilli multiplied rapidly, caseation was widespread, and strong toxic effects were evident.

These tubercle bacilli also possessed a marked capability for spreading about in and overpowering the animal organism into which they were introduced. It is also of interest to notice the differing degrees of susceptibility shown by the rabbits used in this experiment. For instance, two rabbits were inoculated intravenously by Veszprémi with equal amounts of a culture which he designated as culture G. Although both of these test animals developed generalized tuberculosis, one of them died in twenty-eight days as the result of inoculation, while the other one lived for fifty-seven days and was finally killed in order that the extent of its lesions might be determined. As will be mentioned more fully later in this work, this experience of Veszprémi has been often recalled to our minds by the marked and baffling differences of susceptibility shown by animals of similar species, age, and weight for duplicate doses of tubercle bacilli, and indicates the importance of recognizing and accepting the existence of marked differences in individual resistance.

That section of the report of Kossel, Weber, and Heuss[16]—the German Tuberculosis Commission—which records the results of their subcutaneous inoculations of cattle with bovine tubercle bacilli may with advantage be taken up for consideration immediately after this article by Veszprémi. In the commission's experiments (1904) ten cattle were used, with the result that five died of generalized tuberculosis after forty, forty-four, fifty-four, fifty-six, and sixty-five days, respectively. Four were slaughtered after periods ranging from four to seven months. These showed generalized tuberculosis, but the disease was more confined to the lymph glands and had therefore produced a more chronic type of the affection. Finally, the effects of the inoculation of one of the cultures were limited exclusively to the prescapular gland at the site of the inoculation.

Here it will be seen that marked variations may occur in the virluence of tubercle bacilli of bovine origin as well as in cultures derived from human tubercular lesions and sputum. Of the thirty-nine specimens of human tubercle bacilli examined by the commission, four were found virulent for cattle, and those four were all derived from lesions in children.

A review of tests with bovine tubercle bacilli made upon rabbits by Ravenel (1901)[27] shows that his bovine culture T 3 killed the animals in an average time of forty-nine and one-half days, while bovine culture F 6 required one hundred and twenty-six and one-half days on an average for the production of fatal results upon similar animals. That the variations of bovine tubercle bacilli are not limited to virulence is shown by the following statement in the bulletin above quoted:

> In a series of examinations of material coughed up by cows I failed always to find bacilli of what has been described as the bovine type. In fact, the longest bacilli I have ever observed, except in old cultures, were seen in some specimens of this material.

Ravenel has also recorded the infection of three cattle by means of intraperitoneal inoculation with human sputum.

His stand upon the question of the unity of human and bovine tuberculosis is expressed in the following unmistakable lines:[28]

> Although at the time of the London Congress on Tuberculosis I had collected a certain amount of evidence which convinced me of the correctness of my belief in the essential unity of tuberculosis as seen in man and in cattle, and in the intercommunicability of the disease between these two species of mammalia, research in every part of the world has been so stimulated since that time that to-day we are in possession of facts that seem to me to be irrefutable. It appears to me that the case might well rest on the positive evidence afforded already by the discovery of the bovine germ in children.

Schottelius (1903)[29] has reported positive results obtained through feeding human sputum to cattle. The outcome of these cases was such that the writer came to the conclusion that there are no proper grounds for the belief that cases of tuberculosis of cattle due to the ingestion of human sputum at frequent intervals will always heal rapidly and spontaneously. On the contrary, in each of his experiment cattle he found the lesions so general that they must have speedily had a fatal termination had he not slaughtered the animals for purposes of investigation.

By means of his investigations Prettner (1903)[24] has found that typical tubercular lesions follow the inoculation of calves with certain pure cultures of human tubercle bacilli. The surest way of inoculation is intraperitoneal. Cultures of high degree of virulence will here produce acute miliary tuberculosis, in which bacilli are typical, abundant, and virulent.

Mention has been made in previous bulletins of this Bureau of the results obtained by Cippollina, Bang, Orth, Fibiger and Jensen, Karlinski, Thomassen, Arloing, Nocard, Stenstrom, Svensson, Dean and Todd, De Jong, Wolff, De Haan, and Hamilton and Smith, who succeeded in producing well-marked lesions of tuberculosis in cattle with tubercle bacilli derived from man, and in many instances these bacilli were as virulent as those obtained from cattle.

Preisz (1904)[23] found among ten different cultures of human tubercle bacilli none that would produce generalized tuberculosis in cattle. They showed slight variations in virulence, however, as one produced not the slightest lesion, while the most virulent caused an extension of tubercular formation from the point of inoculation to the adjoining lymph glands.

Klebs and Rievel (1902)[14] experienced no difficulty in producing a fatal infection in a calf by causing it to inhale tubercle bacilli derived from a human subject.

The following is quoted from Von Behring (1902):[2]

> In spite of all cultural differences, and in spite of the right material differences in the anatomic appearance of the disease products developed in small and large test animals, we yet have a perfect right to declare that our avian tubercle bacilli and bovine tubercle bacilli are identical species. I mention this because in the following work our original avian culture, and the many modifications of it which we have obtained by its repeated passage through animals, are considered simply deviations of our bovine tubercle bacilli.
>
> I also find that there is less difference between our bovine culture (TB. II) and our human culture (TB. I) than there is between two of our human cultures (TB. I and TB. I*a*). I find that there are cultures of tubercle bacilli coming from man that have but very slight pathogenic power for cattle, and others which are as virulent for cattle, or even more virulent, as many cultures of bovine origin. In direct opposition to the teaching that bovine tubercle bacilli are harmless for man, I have every ground for the statement that bovine tubercle bacilli in general represent a higher grade of virulence than the human tubercle bacilli, and are therefore more dangerous for man.

Although Delépine (1902)[6] has found that human tubercle bacilli are as a rule less virulent than those of bovine origin, he has nevertheless found human tubercle bacilli that were just as virulent as those derived from cattle. He used human sputum in his investigations and produced positive results in cattle in two instances.

Bujwid (1904)[3] reports himself positively decided in opposition to both Koch's and Von Behring's theories. By means of his own experiments he has reached the conclusion that there are different varieties of tubercle bacilli which may reach greater or less degrees of virulence. He found that much depended upon the amount of infectious material which was injected into the system. His tests with cattle proved that they could be injected with human tubercle bacilli, but that the lesions which resulted always showed greater tendency toward localization. He also found that there are differing degrees of virulence possessed by various cultures of avian tuberculosis.

Hueppe (1904) [12] states that tubercle bacilli have developed phylogenetically from saprophytes out of the group to which the actinomyces belong, but he thinks it improbable that any such transformation is occurring at the present time. Their virulence is most varied and is by no means constant even in the same species of animal. The statement that human and bovine tubercle bacilli are entirely different species has been repeatedly disproved. Their differences are only modifications of one and the same species, caused by their adaptation to various kinds of animals. This explanation of variations will also apply to avian tubercle bacilli, they being but forms of the common family. Tubercle bacilli which have become adapted to the tissues of cattle are the most virulent.

Lartigau (1901),[17] starting out with the idea that possibly bacilli derived from different sources might vary considerably in virulence, obtained cultures from various lesions in human beings, such as tuberculous lymph glands, pulmonary tuberculosis, and bone and joint tuberculosis. He found that the morphologic features were fairly uniform. In those organisms in which the virulence was greatest the growth upon culture media was usually least. In one instance a bacillus was obtained from a human being that produced a culture not unlike that described by Theobald Smith as characteristic of bovine tubercle bacilli. This bacillus was excessively virulent and corresponded also in this respect with Smith's description of the bovine bacillus. The inoculation experiments confirm Lartigau's belief that the virulence of the tubercle bacillus varies considerably.

The culture referred to by De Jong [13] in 1902 is worthy of particular attention because of its derivation from sputum; yet notwithstanding this fact it was shown to possess all of the leading characteristics of bovine tubercle bacilli. With this culture he inoculated a calf and a goat intravenously. The calf died nineteen days afterwards with generalized tuberculosis, while the goat succumbed after twelve days, showing tuberculosis of the lungs, liver, and spleen. De Jong concludes from certain of his experiments that while bovine tubercle bacilli are usually more virulent than those of human origin, this is not always the case.

Sturrmann (1903) [33] obtained cultures of the above sputum bacilli isolated by De Jong and by means of continued experiments succeeded in substantiating the statement that they conformed to the bovine type in every important particular.

Among the guinea pigs used in making comparative tests of tuberculins by Wolbach and Ernst (1904) [36] was one series consisting of ten, one half of which were inoculated with human tubercle bacilli and the remaining half under identical conditions with bovine. One of the animals receiving the bovine organism far outlived all of the others, although it finally developed generalized tuberculosis, thus indicating either a remarkable natural immunity of the animal or a lower degree of virulence in this bovine bacillus.

In a later series of experiments Smith (1905)[32] obtained two fresh cultures of human tubercle bacilli, No. XI and No. XII, which were isolated from mesenteric lymph nodes of children. The baccilli of No. XI, when first recovered, were shown microscopically to be as follows: "A few stained solidly; many appeared granular—fragmented. They were frequently in groups of three or more individuals ranged parallel to one another." After passage through a single guinea pig " the bacilli from all cultures examined were plump rods, many of them thick centrally and tapering somewhat toward both extremities, or slightly thicker at one end and tapering toward the other. They were straight or very slightly curved." Measured, when first recovered, they were found to be 2 μ or more in length; after passage through animals they were shorter, being 1 to 2 μ. In pathogenesis culture No. XI approaches but does not quite attain the virulence of fresh bovine cultures. Its morphology is that of the bovine race. Culture No. XII, on the other hand, is essentially of the human type both as to morphology and pathogenesis. It is significant that these two quite divergent races of tubercle bacilli were associated with diseases very much alike in the human subject.

He further states:

> There is no uncertainty as to the capacity of XI to produce genuine tuberculous lesions in cattle. It is not to be denied that its pathogenic power appeared to be somewhat below that of cultures from cattle; but we must here take into consideration the temporary sojourn of XI in the human subject and the possibility of a slight modification therein.

MacFadyean (1903)[18] used both inoculating and feeding tests upon 18 monkeys, and reached the conclusion that monkeys were susceptible to bovine tubercle bacilli either directly or after one passage through the organism of the guinea pig. Animals used in feeding experiments succumbed to general tuberculosis after a like period of time whether the material fed them originated in human or bovine sources. Thus the young monkey proved to be equally susceptible to the human and the bovine strains of tubercle bacilli. The striking difference in the effects produced occurred in the digestive tract. Intestinal lesions were found in the case of every monkey that had been fed with human tuberculous material, while none of the monkeys fed with the bovine material presented any evidence of tuberculous ulcers in the intestine. Within the limits of these experiments it would appear that a food tuberculosis can be as readily brought about by the bovine as by the human strain of the tubercle bacilli in the young monkey.

Chiesi (1904)[4] studied 24 cases of tuberculosis and reached the following conclusions:

> The morphology and chemical characteristics of tubercle bacilli vary in different individuals and at times in the same individual. The morphologic variations in the germ are, as a rule, more numerous and more marked when there is mixed infection. Other things being equal, the severest forms of tuberculosis show, for the most part, short and easily stained bacilli, while the lighter forms show bacilli with extensive morphological changes and poor staining qualities. If kept in the thermostat at 37° C. the tubercle bacillus tends to grow and to change the shape. In sputum kept in this manner we find altered forms next to normal forms. Tubercle bacilli kept in sputum where they occurred become attenuated in the course of twenty days and lose their virulence.

After giving a résumé of the cultural and morphological differences between animal and human tubercle bacilli Beck (1903)[1] writes:

> Comparative tests of both kinds of tubercle bacilli in regard to their pathogenic effect upon various animals show that in general the bovine tubercle bacilli evince a greater tendency toward a generalized attack upon the animal. The dog alone offers an exception to this rule. With this species of animal intra-abdominal inoculations with human tubercle bacilli resulted in an attack upon the lungs in addition to the infection of the abdominal organs, while the lesions resulting from similar inoculation with cultures of the bovine type were limited to an attack upon the abdominal organs.
>
> The animals inoculated with bovine tubercle bacilli died a little earlier than those treated with human tubercle bacilli, and as the lesions in the former gave no evidence of a tendency to remain stationary, it is possible that they would in time have forced their way into the thoracic cavity.

Moeller (1903)[19] found that tubercle bacilli that would grow artificially from sputum without passage through animals were less virulent than those isolated by means of animal passage. He found a marked difference in the virulence of various cultures of human tubercle bacilli, but thought that he was able to account for this through the action upon them of the tissues of the guinea pigs through which they had been passed.

While exhibiting some tubercular tissues which were produced by the inoculation of calves with human tubercle bacilli of a virulent order, Orth (1903)[20] made the following statement:

> The preparations are here again to-day, and you can easily see that the tuberculosis has originated at the point of inoculation, and that the lymph

glands in the second calf also, as in the animal directly inoculated, exhibit the same rapid calcification which is so characteristic of perlsucht; that is to say, their condition does not resemble that common in human tuberculosis, but genuine perlsucht, although the infection was with bacilli derived from man.

Herzog (1903)[11] found that in his experiments relative to the survival of mammalian tubercle bacilli, when introduced into the systems of cold-blooded animals, the same positive results were obtained when bovine tubercle bacilli were used instead of those of human origin.

Pansini (1894)[21] isolated tubercle bacilli having the characteristics of the avian variety from a human being after passing the tuberculous products through guinea pigs. He produced tuberculosis successfully in guinea pigs with avian tubercle bacilli, and says that there can be no doubt of transition forms between the avian and mammalian types.

Phisalix (1903)[22] studied the characters of a culture which he derived from a tubercular intestinal lesion in an emu. This developed both the characters of the avian and of the human types in different cultures upon glycerin-potato. Inoculated into the peritoneal cavity of guinea pigs, it caused the death of these animals in fifteen to twenty days. Cultures grew readily from the lesions of this animal, but at once assumed characters which belong to the human type rather than to the avian. Because of its exceptional virulence for the guinea pig, and of its assumption of the characters of the human tubercle bacilli culturally, Phisalix concludes that the culture in question represents a transformation stage and presents characters of both human and avian types.

Ramond and Ravaut (1898)[26] inoculated frogs with human, avian, and piscine tubercle bacilli, with the result that the avian cultures proved to be the most virulent. The human and piscine forms showed equal virulence.

Through careful investigations Eberlein (1899)[8] has established the fact that tuberculosis of the parrot and tuberculosis of mammals are identical, wherefore he considers the former of considerable importance to man. He found that culturally both grew alike, that microscopically both types appeared alike, and that when inoculated into guinea pigs both produced similar generalized lesions of tuberculosis. He readily infected parrots with tubercular material from man, citing five cases.

Friedmann (1903)[9] gains from his extensive investigations of turtle tuberculosis a few results that are of special interest when applied to the present question of the identity or nonidentity of all types of tubercle bacilli. He used in his work cheesy nodules from the lungs, and had no trouble in obtaining pure cultures from these upon the commonly used culture media at a temperature of 37° C. They showed remarkable similarity to the human type even in the first cultures, and the similarity was enhanced as the result of reinoculation and transfer to new cultures until at last even the most expert specialists in the cultivation of tubercle bacilli were unable to differentiate between them.

While most of the investigations previously carried out with avian tuberculosis have been limited to tubercle bacilli obtained from hens and parrots, Rabinowitsch (1904)[25] was able to work with birds of various kinds and succeeded in isolating 34 different cultures with which to carry out comparative studies. The statement is often made that tuberculosis of the lungs is of very rare occurrence in birds, but out of 55 tuberculous birds this investigator found tuberculosis of the lungs 35 times, and in 5 instances the lesions were limited to an attack of the lungs.

It was also found that the infection of birds did not occur, as had been formerly supposed, only through the ingestion of feces which were contaminated with tubercle bacilli, but that the disease was often spread to birds through the eating of rats or mice which had become spontaneously affected with avian tuberculosis. Weber and Bofinger suggested that these rodents might play an active part in the spread of avian tuberculosis, and Rabinowitsch believes that her experiments have fully proved the fact.

Although avian tubercle bacilli present a peculiar appearance when compared in their manner of growth artificially with that of mammalian tubercle bacilli, Rabinowitsch has found among 34 cultures 2 which grow exactly like mammalian cultures both upon solid and upon liquid media. In addition to these there were a number that approached closely the mammalian type in their manner of growing, and it required continued growing through later generations to prove that they belonged by the majority of their characteristics to the avian type. It has been frequently shown by experiments with bovine and with human tubercle bacilli that there is a great degree of variation of virulence to be found between different cultures derived from tubercular subjects of the same species, and the comparative studies of the avian cultures have proved that such variations of virulence between different cultures of avian tubercle bacilli are also noticeable.

The use of tubercular material direct from birds in the inoculation of guinea pigs gave tuberculosis of equal severity with that resulting from inoculation with mammalian material in several cases, while in other instances the course of the disease was less severe. The use of pure cultures of avian tubercle bacilli for inoculation purposes resulted in a lessening of virulence in the case of tuberculosis produced. Repeated passage of avian tubercle bacilli through guinea pigs failed to enhance their virulence; in fact, some of them became so attenuated by this means that they would no longer produce tubercular lesions in guinea pigs after the fifth or sixth passage. It was found also that certain fishhawks could, like the parrot, become infected spontaneously with mammalian tuberculosis.

Rabinowitsch concludes from these investigations that exchanges of position between avian and mammalian tubercle bacilli are not so infrequent as has been formerly supposed, and the earlier reports of avian tuberculosis appearing in man should therefore be granted greater credence. The results here gained seem to justify one in claiming that avian and mammalian tubercle bacilli are not distinctly separated kinds of bacilli, but only varieties of a single species that have become differentiated through their retention in the bodies of different species of animals.

At the veterinary high school in Hanover various tests were carried out in 1903 and 1904 for the purpose of verifying the results obtained by the German Tuberculosis Commission at Berlin. In his preliminary report of this work Dammann (1904),[6] by whom the tests were made, says:

> The complete report of the experiments of our institute will not be prepared for a few months yet, but if I select one of our cases and give it out for publication now it is because it strengthens with special force the conviction of the possibility of the transmission of human tuberculosis to animals. The case is worthy of the most careful consideration, because it shows that there are cultures of human tubercle bacilli which are not only capable of producing generalized tuberculosis by subcutaneous inoculation upon calves and hogs, but will even produce their death in a short time, exactly as many of the bovine cultures would do.

In a recent address before the Medical Association of Leipzig, Eber (1906)[7] gave the results of a series of experiments which had been carried on under his direction. In this investigation eight cultures of tuberculosis of human origin were tested upon calves ranging in age from two to three months. Of this number two proved to possess only very slight virulence for cattle, two others were of medium virulence, and three were possessed of strong virulence. The eighth culture was found to be without virulence for guinea pigs, and it was therefore deemed useless to test it on cattle. Four cultures derived from cattle were tested upon cattle by methods similar to those used with the human cultures in the same experiment, with the result that two of them were found to be of very slight virulence for cattle, one of medium degree, and only one of a strong degree of virulence. The interesting fact was here demonstrated that it was not an easy matter to cause the infection of cattle with typical tuberculosis by the use of tubercle bacilli derived from cattle, even though the injections were made intravenously.

Goodale (1906)[10] states that within two years he has had under observation seven cases of tuberculous glands of the neck of children from whom he removed the tonsils. On clinical inspection the tonsils showed very little that was abnormal, but on microscopic examination tubercles and giant cells were found. The tonsils were inoculated into guinea pigs, which died subsequently of tuberculosis. Cultures from four of these pigs have been kept under observation by Theobald Smith, who reports that they are cases of bovine tuberculosis. The bacteriology of the cases will be reported in detail by Doctor Smith.

ORIGIN OF CULTURES USED IN PRESENT INVESTIGATION.

The tuberculous material which has been available for the work reported in the present paper has been remarkably varied. There has at no time been a lack of tuberculous tissues from cattle, sheep, or swine, and the supply of similar tissues from human subjects, although not so readily available, has still been ample. The one regrettable feature about the latter material was the inability to obtain as full a history and post-mortem notes on some of the subjects furnishing the cultures for this investigation as could be desired. However, these notes have been made as complete as possible by interviewing members of the families interested, by information gratefully received from the family physicians, or by personal observation.

In addition to the affected specimens from man and from cattle, sheep, and swine, which are commonly made the subject of such comparative studies, many unusual sources have recently been found, and in consequence we have been able to study the characteristics of tubercle bacilli derived from natural infections of the dog, the parrot, the snake, the beaver, the raccoon, the peccary, the agouti, the lemur, the bear, and several varieties of deer and monkeys. The cultures from the wild animals have been recovered more recently and too late to be included in this comparative work; a study of their peculiarly interesting characteristics will form the basis of another paper.

A brief résumé covering the history and such post-mortem notes as were available of the subjects furnishing cultures for this investigation is given below:

1. *Girl I.*—This material consisted of a caseous mesenteric lymph gland of a 6-year-old girl who died of generalized tuberculosis (numerous lesions being found in both the thoracic and abdominal cavities) at the Jefferson Medical College Hospital in Philadelphia. It was impossible to obtain any history of this case except that the child was of sound parentage. The tissue was kindly furnished by Dr. Randle C. Rosenberger, bacteriologist to the hospital, to whom we hereby offer our sincere thanks for this as well as for the material, history, and post-mortem notes of cases designated Girl II, Boy VI, and Case XIV.

2. *Girl II.*—The tissue used in this experiment was obtained from a tubercu-

lar cervical lymph gland in a 13-year-old girl who was successfully operated on at the Jefferson Medical College for scrofula. We are unable to give the subsequent history of the case, as the patient passed from observation.

3. *Sputum A.*—This specimen was recovered from the sputum of Mrs. A., 35 years old, who was suffering from a mild form of pulmonary tuberculosis. The patient removed from the neighborhood immediately after her attack, but is still alive and greatly improved.

4. *Sputum W.*—This sputum was obtained from Mr. W., who removed to Texas in consequence of an attack of phthisis. No report is available as to his present condition.

5. *Boy V.*—The material used for this investigation consisted of the caseous mesenteric lymph gland of a 4-year-old boy who died of generalized tuberculosis. The family history was negative, the other children of the family being entirely healthy. The child developed anorexia, abdominal pains, diarrhea, and abdominal distention, together with a high fever. He had a slight cough, became very much emaciated, and died about six weeks after the development of the symptoms. This child was evidently affected with primary intestinal tuberculosis, as the foci in the lungs were very small and widely scattered and involved only the apices. The spleen and the liver, especially the latter, were likewise sprinkled with tubercular foci. The peritoneum, the omentum, and the mesenteric glands contained many hemorrhagic tubercles, causing numerous adhesions between different portions of the intestines. Four or five ulcers were noticed in the intestinal tract, especially in the vicinity of the transverse colon. Many of the mesenteric lymph glands were swollen and more or less caseated. For this material and the postmortem notes we extend our grateful thanks to Dr. W. R. Brandenburg, formerly on the staff of Garfield Hospital, Washington, D. C.

6. *Boy VI.*—This culture was recovered from the cervical lymph gland removed at the Jefferson Medical College from a 6-year-old boy. The child was suffering with general lymphatic enlargements (cervical, maxillary, and inguinal) for several months preceding extirpation of the cervical nodes; but subsequent history of this case was not obtainable.

7. *BB.*—Students of the comparative virulence of human and bovine tubercle bacilli are already so well informed in regard to this culture through the writings of Ravenel that a detailed description of its origin is quite unnecessary. Suffice it to say that the culture was isolated by Ravenel from the mesenteric gland of a child whose death was due to tubercular meningitis, which was considered to have been of intestinal origin. Our grateful thanks are hereby extended to Doctor Ravenel for permitting the use of this culture in our comparative work.

8. *Sputum C.*—The bacilli of this culture were isolated from the spleen of a guinea pig inoculated with the sputum of a young man 20 years of age, who was in the last stages of acute pulmonary tuberculosis. He was tall and athletic in build and had a negative family history. His health had been excellent until seven weeks prior to death, when he was attacked by la grippe, which developed into pleuro-pneumonia. During the sixth week of illness tuberculosis was suspected, and a sample of sputum was brought to one of us for examination. Six days later death occurred from phthisis florida. No autopsy was made.

9. *Sputum E.*—The material from which a culture was obtained for this study consisted of sputum from Mr. E., colored, an employee of this Department, who is suffering with chronic pulmonary tuberculosis. He comes from a tuberculous family and has shown symptoms of the disease for the past three years, although at present in a fairly good state of nutrition.

10. *Lung X.*—This specimen was obtained from the lung of a cadaver which was a subject for dissection at a local medical college. Death was due to cerebral apoplexy. The subject appeared to be that of a 55-year-old man, whose previous history was not available. Many foci of tuberculosis were found in both lungs, but were quite scattered and evidently bore no relation to the cause of death. The cheesy pus from a nodule the size of a hazelnut in the apex of the right lung was the material used in obtaining the culture.

11. *Case XI.*—This culture consisted of tubercle bacilli obtained from the pus of an umbilical fistula in Mrs. J. The wound started as a small abscess at the lower border of the navel, which was evacuated and treated aseptically. After three months a similar development occurred which, despite a repetition of the above treatment, persisted for several months during the summer, but it was finally healed. Its recurrence four months later was the cause for a radical operation. The patient was etherized and the abscess, together with 1¼ inches of the fistulous tract extending into the abdominal muscles, but not involving the peritoneum, was excised and the wound sutured. No recurrence has thus far occurred, nor is there any indication of tuberculosis elsewhere in the body. The family history is negative, and the patient appears in robust health. In the opinion of the family physician the case must be considered as one of localized infection.

12. *Sputum D.*—This culture was obtained from the sputum of Mr. D., 60 years of age, whose attack with caseous pneumonia about twelve years ago resulted in a small cavity formation in one lung, together with pleuritic adhesions. Several years ago, after an attack of bronchitis, mucopurulent expectorations occurred, associated with more or less hemorrhage. An examination of a small necrosed piece of tissue which was expectorated during a paroxysm of coughing revealed tubercle bacilli, which were obtained on blood serum in pure culture from the spleen of an inoculated guinea pig. The patient three years ago removed to a high, dry altitude for the benefit of his health, and during the past year three microscopic examinations and inoculation tests of his sputa have resulted negatively in each case.

13. *Sputum H.*—This material consisted of sputum from Mr. H., 53 years of age, who was in an advanced stage of chronic pulmonary tuberculosis. The patient had been ill for the past six years, and on examining the sputum myriads of tubercle bacilli were observed. He continued to grow worse until death occurred, seven months after the receipt of his sputum. No autopsy was performed.

14. *Case XIV.*—The culture recovered for this study was obtained from a mesenteric gland of a 23-year-old man, a pauper, who died at the Philadelphia hospital from generalized tuberculosis. It was impossible to obtain any previous history of this subject. The post-mortem examination showed the lungs to be permeated with numerous areas of tuberculosis, as were the bronchial and mediastinal lymph glands. The liver likewise showed a few isolated foci. The small intestines contained several tubercular ulcers elongated transversely to the axis of the intestines, these lesions being evidently secondary to the pulmonary lesions. Several nodes of the chain of the mesenteric lymph gland were enlarged and more or less caseated. One such gland formed the sources of the emulsion used in the inoculation of a guinea pig, from whose spleen a pure culture on dog serum was obtained.

15. *Case XV, a and b.*—These specimens were isolated from the urine of a 32-year-old man at two periods five weeks apart. The man, who was affected with pulmonary tuberculosis, sought relief from an intense pain in the lumbar region, irregular fever, and slight cachexia. Tuberculosis of one or both kidneys was suspected by his physician, Dr. C. McPherson, to whom we extend thanks

for the material furnished. Culture *a* was derived from the urine voided from the bladder, while culture *b* was obtained from the bloody urine secured by catheterizing the right kidney. The urine from the left kidney failed to show any tubercle bacilli. No further history of this case has been obtained.

16. *Sputum B.*—This sputum was obtained from Mr. B., 21 years-old, who was affected for two years with pulmonary tuberculosis. His mother, a brother, and a sister had died of the disease, and another sister was similarly affected. This patient is still alive, and seems to be in better health after his sojourn for two years in the mountainous region of North Carolina.

17. *Sputum G.*—The culture used in this experiment was recovered from the sputum of Miss G., 18 years of age, whose family history is negative. Miss G. is affected with pulmonary tuberculosis, and is gradually growing worse, despite the best of home treatment.

18. *Sputum L.*—This material consisted of sputum from Miss L., 22 years of age, who died of acute phthisis five months after the receipt of this sputum. No autopsy was held.

19. *Monkey.*—This culture was recovered from the spleen of a guinea pig inoculated with an emulsion of the lung of a monkey (rhesus). About November 17, 1901, a large tubercular abscess containing pus appeared in the submaxillary region, which was lanced by a veterinarian and the pus scraped out. The monkey was treated by giving cod-liver oil and provided with a large, well-ventilated cage, receiving the best of attention. The principal diet consisted of fruits, and the animal was particularly fond of bananas, hemp seed, and sunflower seed. At no time was it fed on meat, and it received only two bottles of sterilized milk just before death. While sick it drank considerable water and gradually wasted away, and declined in health until death resulted on February 15, 1902. The creature was 2 years old and weighed 10¼ pounds. Postmortem examination showed enlarged caseated right retropharyngeal lymph gland and extensive tuberculosis of the lungs, with involvement of the bronchial and mediastinal lymph glands. The liver also contained a few areas of tuberculosis, and several mesenteric lymph glands were swollen, with cheesy foci present. No ulcers were observed in the intestines.

20. *Bovine S.*—This culture was obtained from the lymph gland of a guinea pig which had been inoculated with a portion of a mesenteric gland of a cow condemned for generalized tuberculosis at a Philadelphia abattoir. On postmortem examination the lungs were found to be studded with areas of tuberculosis varying in size from a hazelnut to a hen's egg, many of which had coalesced into larger clumps of affected tissue. The entire chain of mediastinal lymph glands was swollen and caseo-calcareous, while the bronchial glands were distinctly gritty, dry, and enlarged. The liver contained a few yellowish foci, while several mesenteric glands were swollen and cheesy. A few grape-like tubercles were found on the omentum and attached to the peritoneal side of the diaphragm. The carcass was poor in nutrition and was that of a cow about 10 years of age.

21. *Bovine T.*—This material was kindly furnished by Dr. J. P. Turner, veterinarian of the District of Columbia health office, to whom we extend our thanks. It consisted of the lung tissue of a young cow 5 years old that was condemned for pulmonary tuberculosis at Benning, D. C. Tubercular lesions were found scattered through both lungs, causing almost a complete solidification of the superior border of the left principal lobe. The bronchial and mediastinal glands were likewise involved, especially the posterior mediastinal, which was as large as a goose egg and caseo-calcareous.

22. *Bovine F.*—This culture was obtained from a tubercular abscess found in

an excavation of the body of one of the cervical vertebra of an aged cow slaughtered at South St. Joseph, Mo., and condemned as unfit for food owing to the generalized and extensive tubercular lesions present. The cervical and thoracic lymph glands were all involved, while both lungs contained innumerable areas of tuberculosis. The serous membranes lining the thoracic and peritoneal cavities showed irregular clusters of pearly bodies scattered unevenly over their surfaces, and a few tubercular areas were also found in the liver and in several mesenteric lymph glands. The previously mentioned abscess of the cervical vertebra was observed on splitting the carcass.

23. *Bovine Y.*—The tissue from which this culture was obtained consisted of a mesenteric lymph gland of a cow about 8 years old affected with extensive and generalized tuberculosis. The retropharyngeal, bronchial, and mediastinal lymph glands were all enlarged and more or less calcareous. The lungs contained many isolated and confluent zones of tuberculosis, at least two of which were in direct communication with the bronchi. The liver showed six foci of tuberculosis, while the surface of the spleen was sprinkled with several nodular masses of tubercular tissue hemorrhagic in character. Two mesenteric lymph glands were slightly enlarged and showed several hemp-seed-like foci of caseous material containing tubercle bacilli.

24. *Hog O.*—This culture was recovered from the spleen of a guinea pig inoculated with a mesenteric lymph gland of a hog which was condemned on post-mortem examination at a Chicago abattoir for generalized tuberculosis. The cervical, bronchial, and many mesenteric lymph glands were tubercular. The lungs contained miliary foci of tuberculosis. The liver and the portal lymph gland showed foci of tuberculosis, while the spleen was enlarged to twice its size and contained numerous isolated areas of tuberculosis throughout its parenchyma. The hog was about 1 year old and was received from the West, but whether it had been fattened on the product of the dairy or otherwise was impossible of determination.

25. *Hog K.*—This material was received from an abattoir in Quincy, Ill., and consisted of a submaxillary lymph gland from a hog otherwise unaffected with tuberculosis. The hog was young, in good condition, and the carcass passed for food. No information as to its origin or previous existence was available.

26. *Hog St. E.*—This tissue consisted of the gastric lymph gland of a hog which had been fed by Dr. E. C. Schroeder, superintendent of the Bureau Experiment Station, on the milk of cow 74, from the St. Elizabeth Asylum herd of cattle used in a previous milk-feeding test by this laboratory. The hog began feeding on the milk November 23, 1900, and continued to receive this food until its death, May 2, 1901. The post-mortem by Doctor Schroeder showed the carcass to be in good condition. The lungs contained many tubercular foci, several of which had become confluent. The mediastinal glands were much enlarged and contained many necrotic points of a tubercular character. The liver was well sprinkled with tubercular foci, while the gastric glands were much enlarged and on section were found to contain a large number of tubercular points of a caseous character. Several tubercular nodules were observed on the parietal pleura.

27. *Sheep M.*—This culture was obtained from the superficial inguinal lymph gland of a guinea pig injected with a portion of a tubercular mesenteric gland of sheep 57 which had been born and raised at the Bureau Experiment Station. This sheep had been used in an experiment with a venereal disease of horses, and showed on post-mortem examination three tubercular mesenteric lymph glands. No other lesions of tuberculosis were detected. This was evidently a case of natural infection through the digestive tract.

THE CULTURES SELECTED FOR THE TESTS.

In the comparative study of the above material no attempt has been made to carry each culture through all the species of experiment animals which were used with some of the cultures. After studying their growth and morphology and inoculation results on guinea pigs and occasionally on rabbits and cats, the cultures were grouped as a matter of convenience for further investigation, and a representative of each group was carried along and used in the inoculation experiments on larger animals. Thus this preliminary work indicated that there was no appreciable difference between eight of the sputum cultures, the two specimens of the urine culture, and the monkey culture, and therefore the latter culture was selected for continuous work, inasmuch as the remaining sputum culture (C) possessed characteristics somewhat unlike the others, and for this reason was retained for more extended study. Of the eight cultures from tubercular human tissues, BB culture was used to compare results with those of Ravenel, Boy V culture was retained owing to its greater virulence, while cultures Girl I and Case XIV were taken from the six remaining cultures owing to their being considered fit representatives of this group.

This method was adopted on account of the desire to obtain as many cultures as possible for comparison, as it was thought needless to carry so many different organisms through a series and subseries of cultures and of various animals, when they could be grouped tentatively and a representative selected for more detailed investigation. That this is not the most desirable method to pursue will be shown by reference to Case XIV culture, which, although at first similar in growth, morphology, and virulence to Girl I culture of this group, later showed characteristics peculiar to itself, especially with reference to its virulence for dogs. It is probable that others might have possessed similar variations from the ordinary if carried through many species of animals, but such work would have been too extensive to have been performed successfully along the lines we have followed, although it opens the subject of the possibility of determining many points of variation more or less persistent between tubercle bacilli of similar origin if injected into many species of animals.

In selecting a bovine culture for comparative work, the pathogenesis of hog cultures O, K, and St. E, and sheep culture M, and the bovine cultures F, Y, and T indicated that they were all of a high virulence and probably all of the bovine type, so that Sheep M and Hog O were taken as representatives, not only because of their similarity in morphology, cultural characteristics, and pathogenesis to the bovine organism, but furthermore on account of their origin in different species—sheep and hog. The remaining bovine culture—S—was retained for a more exhaustive study owing to the greater ease and rapidity with which it developed on dog serum and to its milder virulence for rabbits than was shown by the other bovine cultures.

All the above cultures were isolated at various times within twenty-one months, but those carried through the entire experiment were practically of the same age, the isolation of all falling within a period of fourteen weeks. This similarity in age was also a factor, other things being equal, in selecting the representative culture.

Cultures from a dog, a parrot, and several wild animals have been recovered more recently, and although too late to be included in this comparative work, a familiarity with their characteristics has no doubt assisted in extending our knowledge upon which to base general conclusions.

TECHNIQUE OF THE EXPERIMENTS.

ISOLATION OF CULTURES.

The methods employed in obtaining uncontaminated cultures of tubercle bacilli from the original tubercular specimens and the media used for the development of these cultures were as similar to those suggested by Smith in his comparative study of tubercle bacilli as it was possible to adopt.

In order to procure pure cultures from the various tubercular fluids (sputa, pus, and urine), suspensions in physiologic salt solutions were obtained and inoculated subcutaneously into guinea pigs ranging from 330 to 480 grams in weight. In the case of tubercular tissues small sections of the affected material were placed, with aseptic precautions, beneath the skin of guinea pigs. As a result of the protective agencies of the body of the animal, contaminating organisms were usually eliminated before the more virulent and slower-growing tubercle bacilli had become fully established. This mode of separation has given very satisfactory results. The guinea pig is so susceptible to all forms of tuberculosis that it sooner or later gives evidence of the encroachment of the disease, no matter which type is being tested. These animals may be chloroformed to prevent secondary infections as soon as they begin to give evidence of wasting, which was the rule in our experiments, or they may be kept until the disease terminates fatally.

With the more virulent bacilli this loss of flesh was observed about the third week, while the less pathogenic organisms required five or six weeks and sometimes longer. At the necropsy bits of tubercular tissue, usually from the spleen or a distal lymph gland, were removed under the strictest aseptic precautions and transferred to culture tubes containing previously prepared dog serum. The blood was obtained from the femoral artery of healthy dogs by the observance of the usual aseptic measures. The animal is etherized and the artery is exposed and punctured by a sterile glass rod conveniently bent, through which the blood is conducted into sterile Erlenmeyer flasks. The serum expressed from the resulting clot is drawn up in sterile pipettes, distributed into Salmon tubes, and hardened in a serum oven at 76° C. on three successive days. These tubes are always kept inclined at an angle of about 10°. The resulting medium is not as hard and dry as when a higher temperature is used, nor is it so soft as to be easily broken when sown with tubercular material. After the first two weeks of incubation the tubercular tissue on the serum is crushed against the side of the tube with a heavy flat platinum needle and spread over the surface of the medium for the purpose of starting fresh colonies. Whenever these procedures result in a pure growth of the tubercle bacilli upon the serum the investigator is supplied with the desired culture, and he can then proceed to take up his comparative study of the organism. For the purpose of providing uniform conditions, one incubator only was used, and where possible the transfers, which were made every four to six weeks, were inoculated into tubes containing media from the same batch of serum. The incubator was kept at from 37° to 38° C. and contained a vessel of water to preserve a moist atmosphere.

PREPARATION OF SUSPENSIONS FOR INOCULATIONS.

The difficulty in obtaining a method by which a uniform quantity of tubercle bacilli will be injected into each species of experiment animals is manifest, but the use of a suspension of an opacity similar to that possessed by a 24-hour-old peptone culture of typhoid-fever bacilli has been adopted, as suggested by Smith. When the tubercle bacilli have developed sufficiently on the dog-serum medium certain portions of the surface growth are scraped off by means of a stout, flat, platinum needle and transferred to a dry sterile tube. The masses of growth are minutely broken up against the sides of the tube, which process is aided by an occasional drop of sterile fluid. When well divided sufficient physiologic salt solution is added to make the resulting mixture conform to the cloudiness possessed by the standard typhoid-fever culture. After the heavier particles have settled, the supernatant fluid is used for inoculation. As a further check on the quantity of tubercle bacilli injected, cover-slip preparations were always made to determine more accurately the degree of pollution present. This method was always adhered to with all culture inoculations which were used in the first series of the various species of animals inoculated. An attempt was made to continue the inoculation of culture suspension into the subseries of animals, but the time consumed in awaiting the development of colonies, the failure of some tubes to show growth, and occasional contaminations indicated that this course, while highly desirable, was rather impracticable, and several times would have resulted in the loss of the particular organism had not inoculation of animals been made simultaneously with the transfer on culture media. For this reason inoculation of animals after the first series was made by making an emulsion in salt solution of tubercular tissue from the spleen, a distal lymph gland, or other metastatic lesions, in order to insure against any contaminating organism; and freedom from contamination was always determined, so far as a microscopic examination permitted, before the injection.

DOSAGE.

In selecting the doses to be given to the various species of experiment animals an endeavor was made not only to give a uniform quantity of tubercle bacilli to each species, but to give well-proportioned amounts also. Thus cattle received subcutaneously 4 c. c. of either the culture suspension or the tissue emulsion, 2 c. c. being injected in front of each shoulder. This location was selected because of the advantage to be derived from noting whether the bacilli spread from the seat of inoculation to the adjacent prescapular lymph glands. The gradual enlargement of these glands is an indication of the virulence of the injected organism. As is well known, bovine tubercular lesions frequently fail to reveal as many bacilli on microscopic examination as similar human lesions show. This condition of sparsity of bacilli occasionally happened when it was desired to obtain the definite amount of tubercle bacilli in an emulsion from a preceding case of tuberculosis, especially a case inoculated with bovine tubercle bacilli. In some cases it was necessary to examine emulsions from several lesions at different locations before a fluid sufficiently polluted with the virus could be obtained, while in other cases, although the usual quantity of suspension had been injected, the total number of bacilli present was lower than the standard. This condition may account for several apparent discrepancies which will be referred to further on. Goats and sheep were similarly injected with 2 c. c. of tubercular fluid, 1 c. c. in front of each shoulder. The failure to obtain enough goats for this experiment necessitated the substitution of sheep for the subseries of inoculations. Dogs were inoculated with a like quantity, in a similar location. Cats received 1 c. c. of the virus, some subcutaneously, but the majority intrathoracically. Rabbits were injected with 0.5 c. c. subcutaneously, on the side of the chest, a few being injected with this size dose intravenously. Mice received 0.1 c. c. of the tubercular virus subcutaneously, at the base of the tail.

STAINING OF BACILLI.

In all of the work of preparing mounted specimens for examination during this investigation the aim has been invariably to stain in a uniform manner all slides that are to be examined comparatively. Carbol-fuchsin has always been chosen as the bacterial stain, and methylene blue has been relied upon for counter staining. In staining tissue for histological study the sections were placed in a large watch glass, covered with cold carbol-fuchsin, and allowed to remain overnight at room temperature under a bell jar. The following morning they were removed from the stain and placed for two minutes in a 20 per cent aqueous solution of sulphuric acid. They were then counter stained with alkaline methylene blue, dehydrated in alcohol, cleared with xylol, and mounted in Canada balsam. In staining bacilli from the various cultures or from lesions of the experiment animals in cover-glass preparations, a small quantity of the surface growth from each of the tubes containing bacilli to be compared, or a similar quantity of tuberculous tissue, is placed upon a separate cover slip and spread out thinly and evenly. After being allowed to dry under a bell jar at room temperature it is fixed by flaming, and carbol-fuchsin is then applied for one-half hour. It is then decolorized by a 20 per cent aqueous solution of sulphuric acid from ten to thirty seconds and counter stained in alkaline methylene blue. Comparisons were always made between bacilli of the same age of development, generally taken from the surface of the same batch of serum, fixed and stained at the same time and by the same methods, thus reducing artificial variations to a minimum.

APPEARANCE OF CULTURES ON DOG SERUM.

Cultures of mammalian tubercle bacilli when grown upon dog serum present at the beginning either a fine, thin, veil-like growth which extends and thickens more or less rapidly toward the formation of a whitish concretion which may assume later the appearance of warty, scale-like, or wrinkled masses, or the original growth appears as delicate, scattered points which gradually extend and increase in size until they may finally coalesce into masses similar to those resulting from growths which were first of the appearance of thin layers. In either case the typical culture of mammalian tubercle bacilli gives no suggestion of a slimy or viscid mass, such as is often produced by avian cultures.

The early growths from tubercular tissues frequently appear very tardily, and their growth for a few generations is feeble and reluctant. As a rule cultures which develop but tardily and scantily will be found possessed of virulence greater than that held by cultures of prompt and luxuriant development, and if submitted to microscopic examination the individuals will commonly be shown as short, straight, evenly staining rods. Hence the culture presenting on serum a scanty, tardy growth may be considered as being culturally representative of the bovine type, while the one which exhibits a rapid, luxuriant multiplication may be taken to represent the usual cultural attitude assumed by the human tubercle bacillus sown upon solidified serum.

Our Girl I culture, and the others examined in this test, of which this was selected as a representative, grew rapidly and bulkily when first recovered from the animal tissues. Subcultures from Girl I made perceptible increase in size by the fourth or fifth day, and gradually extended all over the surface of the medium, presenting a white, granular appearance. Cultures recovered after passage through the rabbit were similar to the original cultures in appearance, and after artificial cultivation for three years the most marked changes were a tendency toward a more pronounced grouping or heaping up in the manner of growth, and to a less diffuse vegetation.

The Boy V culture presented quite a contrast to the culture just described, in that it grew very slowly at first and only acquired a capacity for rapid multiplication on serum after three or four generations had been obtained. Even at its best it would not produce such thick masses of substance as were grown by the Girl I culture, but developed upon the surface of the culture serum as a thin whitish layer, thickly sown with small globules or granules. After repeated passage through a series of dogs and of cats the cultures recovered in both instances from these animals showed very plainly an increased tendency toward the production of large, white colonies raised above the surface of the serum, as though the addition of material to the little mounds or heaps was being gradually forced into them from beneath. Cultures recovered after passage through cattle and sheep were very similar in appearance to the original stock culture and corresponded to the bovine cultures upon serum.

Culture BB, isolated by Ravenel, showed practically no difference from culture Hog O in its cultural appearance. The culture received at this laboratory was the twelfth generation, and had already become accustomed to growth upon artificial media to such an extent that subcultures showed an increase of substance by the eighth day after transfer. The passage of BB through the cow, the cat, and the sheep produced no appreciable alteration in its mode of growth, and at all times the cultures recovered from these test animals showed close relationship to cultures which manifested the typical bovine appearance.

Culture Sputum C made only a very reluctant growth when first isolated, requiring three weeks or more for evidence of growth in subcultures. Later subcultures grew more readily, except those recovered after passage through cattle and sheep. These presented very tardy growths for more than a year after being recovered and isolated, always showing a thin, veil-like form of growth, with a sprinkling of minute white granules scattered over its surface. These bore a very close resemblance to typical bovine cultures.

Case XIV has produced a wide variety of cultures, so far as macroscopic appearance is concerned. Instead of growing in dry, scaly masses, as tubercle bacilli of the human type are accustomed to do, this particular culture has shown more or less of a tendency to spread out over the surface of the culture media as a moist, uniform growth, somewhat after the manner of avian cultures, although it constantly refused to grow with the luxuriance which is usually observed in cultures of the avian type.

The monkey culture was at all times true to the characters of human cultures. The original growth appeared on the sixth day and developed in dry, scaly masses of whitish color and considerable depth. Transfers to subcultures showed prompt and luxuriant development.

Culture Bovine S was at all times a reluctant grower. Although considerably less virulent than Bovine T culture, it proved, contrary to our expectations, to be much more difficult to cultivate. It required almost five weeks for the original growth to develop. When successful cultures were obtained they appeared as fine, disconnected granules of slow development. Subcultures remained apparently inert for three to four weeks, even after many generations.

Hog O and Sheep M cultures were very similar in appearance to each other, and also to Bovine T culture. They all grew promptly in subcultures after the original growths were obtained, and produced fine, granular colonies of small size, scattered over the surface of the solidified serum in great profusion. The typical "ground-glass" appearance so frequently noted in the mention of bovine cultures was plainly in evidence in all of the cultures of these three varieties.

A careful examination of numerous cultures and subcultures of the above organisms, covering many generations during a period of four years, has resulted in the classification of these growths into three classes. A study of these gross appearances was always made upon cultures of the same age, inoculated with a relatively similar quantity of organisms upon the same batch of dog serum, kept in one incubator under precisely the same conditions.

The first class includes those cultures which produce on the surface of dog serum a thin, dry, scanty growth which has been described as peculiar to bovine bacilli, and contains human cultures Boy V, BB, and Sputum C, and bovine cultures S and T, Hog O, and Sheep M. It will be observed, however, that Sputum C culture does not conform in morphology and virulence to the bovine type, although it was found to have far greater pathogenicity than is usual for sputum bacilli.

DESCRIPTION OF PLATE I.

FIG. 1. Human tubercle bacilli, Boy V. Dog-serum culture, second generation, 19 days' growth.

FIG. 2. Human tubercle bacilli, BB. Dog-serum culture, twelfth generation, 19 days' growth.

FIG. 3. Human tubercle bacilli, Boy V. Dog-serum culture, sixteenth generation, 19 days' growth.

FIG. 4. Human tubercle bacilli, Boy V. Dog-serum culture, first generation, 19 days' growth, after passage through sheep No. 104.

FIG. 5. Human tubercle bacilli, Boy V. Dog-serum culture, fifth generation, 19 days' growth, after passage through 15 cats.

FIG. 6. Human tubercle bacilli, Boy V. Dog-serum culture, second generation, 19 days' growth, after passage through 5 rabbits.

DESCRIPTION OF PLATE II.

FIG. 1. Human tubercle bacilli, Case XIV. Dog-serum culture, second generation, 18 days' growth.

FIG. 2. Human tubercle bacilli, Case XIV. Dog-serum culture, second generation, 18 days' growth, after passage through sheep.

FIG. 3. Human tubercle bacilli, Case XIV. Dog-serum culture, first generation, 18 days' growth, after passage through 3 dogs.

FIG. 4. Bovine tubercle bacilli, Sheep M. Dog-serum culture, original culture, 23 days' growth.

FIG. 5. Bovine tubercle bacilli, Sheep M. Dog-serum culture, second generation, 23 days' growth, recovered from the seventh of a series of rabbits.

FIG. 6. Bovine tubercle bacilli, Sheep M. Dog-serum culture, fourth generation, 23 days' growth, after passage through a series of 6 cats.

DESCRIPTION OF PLATE III.

FIG. 1. Bovine S tubercle bacilli. Dog-serum culture, first generation, 19 days' growth.

FIG. 2. Bovine S tubercle bacilli. Dog-serum culture, thirteenth generation, 19 days' growth.

FIG. 3. Bovine S tubercle bacilli. Dog-serum culture, third generation, 19 days' growth, recovered from sheep No. 117.

FIG. 4. Bovine S tubercle bacilli. Dog-serum culture, first generation, 19 days' growth, after passage through 3 rabbits.

FIG. 5. Bovine tubercle bacilli, Hog O. Dog-serum culture, eleventh generation, 19 days' growth, after passage through 5 cats.

FIG. 6. Bovine tubercle bacilli, Hog O. Dog-serum culture, first generation, 19 days' growth, recovered from the seventh rabbit in a series.

These cover-glass preparations were stained with carbol-fuchsin and decolorized with 20 per cent sulphuric acid. Drawn with camera lucida at base of stand; Zeiss 2 mm. oil immersion objective and No. 4 compensating ocular, ×900.

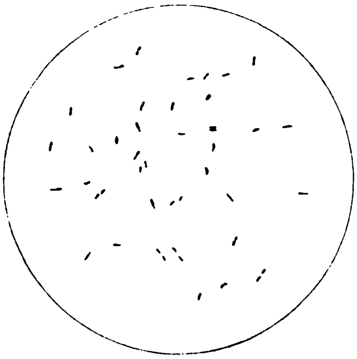


FIG. 1.

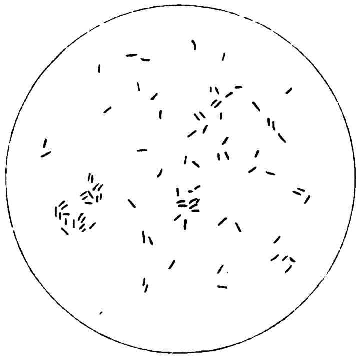


FIG. 2.

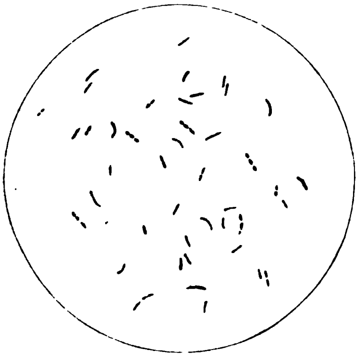
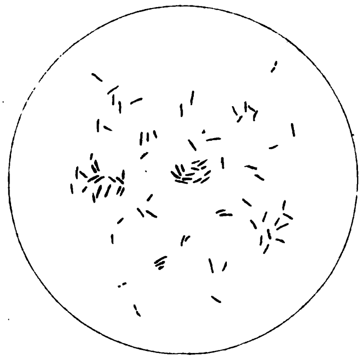


FIG. 3.



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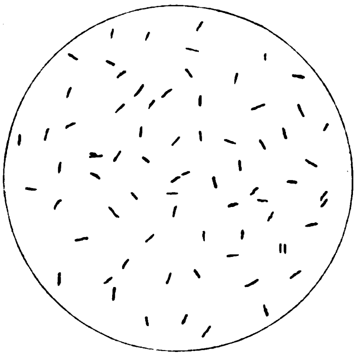

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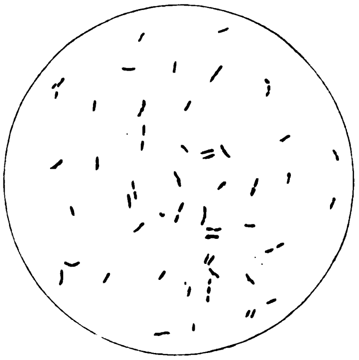


FIG. 6.

HAINES, DEL.

A. HOEN & CO. BALTIMORE.

VARIOUS FORMS OF HUMAN TUBERCLE BACILLI.

Bul. 96, Bureau of Animal Industry, U. S. Dept. of Agriculture.

PLATE II.

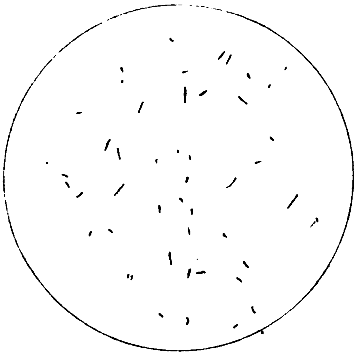
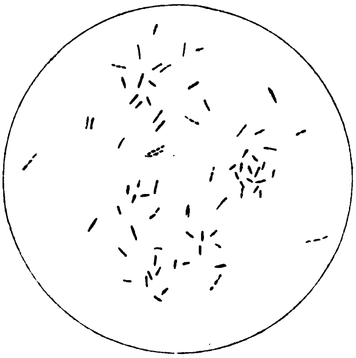


FIG. 1.



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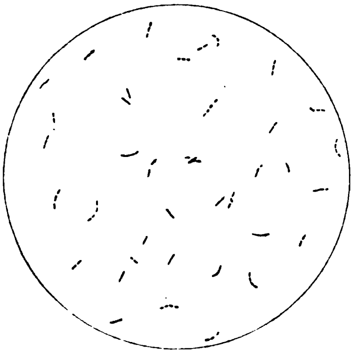

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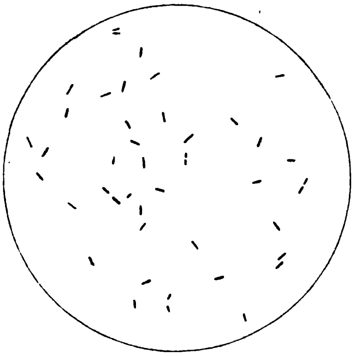

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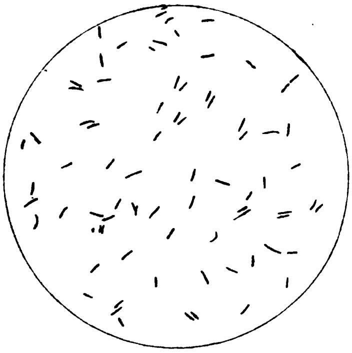

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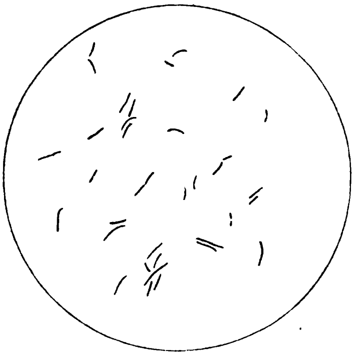


FIG. 6.

HAINES, DEL.

A. HOEN & CO. BALTIMORE

VARIOUS FORMS OF HUMAN AND BOVINE TUBERCLE BACILLI.

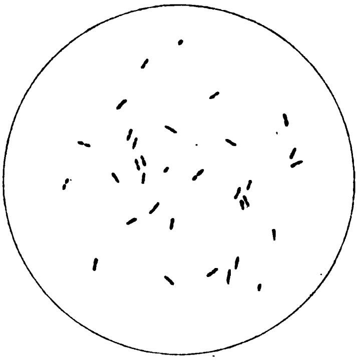
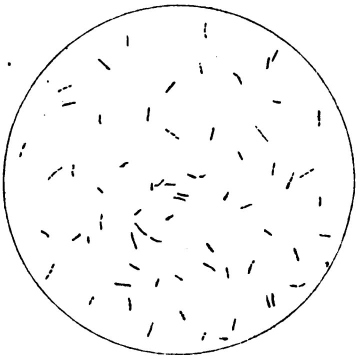


FIG. 1.



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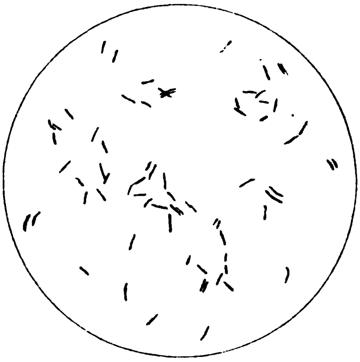

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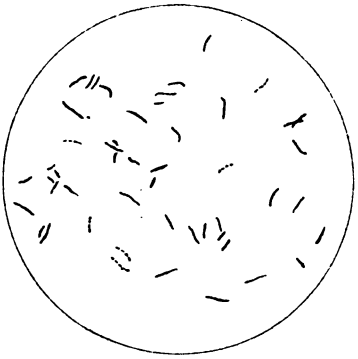

FIG. 4.

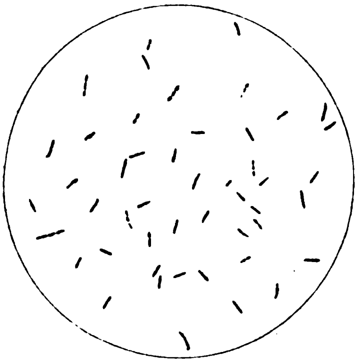

FIG. 5.

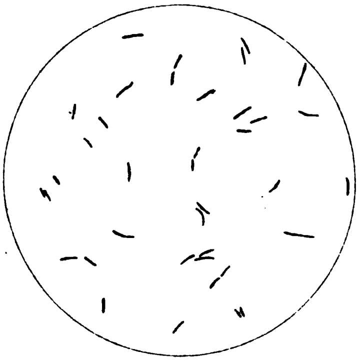

FIG. 6.

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VARIOUS FORMS OF BOVINE TUBERCLE BACILLI.

The second class presented a profuse growth of quickly appearing colonies, which soon piled up over the surface in luxuriant development similar to cultures described as human types. These cultures include those represented by culture Girl I and the monkey culture.

A third class consisted of one member, Case XIV, which, as described above, showed a moist growth somewhat similar to that accredited to avian bacilli. This culture, however, did not coincide with the avian type in any other respect.

CHARACTERISTICS IN SUBCULTURES.

Judging from the naked-eye appearance of subcultures of the different varieties of tubercle bacilli studied, it may be said that the most noticeable change as one passes away from the original culture is an increased adaptability to growth and multiplication under artificial conditions. The perceptible increase of bulk occurs earlier in the later subcultures than it did in the original, and the bulk finally attained in each tube will, with thrifty cultures, show gradual accession.

MORPHOLOGICAL EXAMINATIONS.

The form assumed by tubercle bacilli when grown upon solidified dog serum is at the present day one of the leading factors in their classification as representatives of a particular type. The statement that a certain culture of tubercle bacilli is of the bovine type does not necessarily mean that it was obtained from some animal of the bovine race, but gives expression to the fact that when examined microscopically the bacilli forming such culture are presented to view as short, straight, uniformly stained rods. Reference to Plate I, figures 1 and 2, will show the morphological character of tubercle bacilli of the bovine type, even though the cultures here represented are of known human origin. On the other hand, tubercle bacilli that are strictly of the morphology of the accepted human type may be met that are traceable to bovine origin.

Girl I culture and the others of its class produced individuals that were 3 μ in length, beaded and slightly curved, with lesser members down to not over 1 μ in length, and that stained evenly. Different slides show a variation in the proportion of long or short forms, but the above extremes show no material deviation. Continuous cultivation of this organism by subcultures on dog serum has failed to affect materially the morphology of this bacillus. Repeated passage of this culture through guinea pigs and rabbits has failed to produce any noticeable alteration in the form of its members, unless it may have been to add slightly to their thickness.

Culture Boy V was a good exponent of the bovine type of tubercle bacilli from the start. Measuring from 1 μ to 1.5 μ in length, its individuals were found to be plump, straight rods with even staining. (Pl. I, fig. 1.) Continuous cultivation upon dog serum has apparently given to the individuals of this culture a tendency toward the attainment of slightly greater length. (Pl. I, fig. 3.) In old cultures after the seventh generation they show a greater readiness to produce longer forms than is possessed by the culture Bovine T grown under similar conditions, but no greater than culture Sheep M has shown. Passage of tubercle bacilli from culture Boy V through cattle does not seem to have any effect upon their form. Passage through sheep gives them a slight permanent elongation, without beading. (Pl. I, fig. 4.) On their recovery from the organs of cats or rabbits the individual bacilli are seen to be transformed completely; they are much longer and much slenderer, uniformly beaded, and frequently curved. Returning them to the surface of dog serum they lose much of their added length, but still possess a greater length and more beading than the members of the original stock culture. (Pl. I, figs. 5 and 6.)

Continuous cultivation of Culture BB for three years upon dog serum has not greatly changed the individual micro-organisms, except to make them a trifle more slender. This culture, like the one described above, became greatly elongated by passage through the cat and the rabbit when obtained direct from the tissues, but later cultivation served to shorten them again almost to the starting point.

The statement has been made in the introductory part of this work that culture Sputum C possessed characteristics somewhat different from those of the other sputum cultures tested. When first recovered in pure culture the members were noticeably short for sputum bacilli—1 to 2 μ—and there was also an unusual proportion that stained evenly, showing no beading. This characteristic has been lost somewhat during its long cultivation. Beaded forms have come to predominate, increased length is shown, and a greater proportion of the members show curves.

Passage through cattle, sheep, and dogs seemed to fix the short, straight form more permanently upon the bacilli, and subcultures from these cultures showed little inclination to elongate or to become beaded until several renewals had been reached. After passage through cats and rabbits an increase of length and of beading could be determined, which did not entirely disappear upon further cultivation.

The individual bacilli from cultures of Case XIV can not be said to have adhered to any particular type, owing to the intermixture of numerous forms, broader in the center than at the ends, with others which may be said to represent both the bovine and the human types. The major part of the bacilli shown upon the slides are slender rods, showing a slight tendency toward beading and curving. A few long individuals are also shown, and some short members may be seen in most of the slides examined. (Pl. II, fig. 1.)

Passage through the cow or the sheep does not entirely eliminate the short, irregular forms, but it does remove most of the long, beaded bacilli. (Pl. II, fig. 2.) Recovered from dogs, they appear as very delicate, slender, beaded threads, intermingled with short, evenly stained organisms. (Pl. II, fig. 3.) Further cultivation upon dog serum does not fully remove the variety of forms which are to be found in early cultures. In the body of the cat the bacilli acquire a more uniform length. This culture is somewhat at variance with the others studied, in that it possesses no form or length that may be considered typical.

The monkey culture gave the appearance at all times of a typical sputum culture. The individual bacilli were long, beaded, and often curved. It retained its characters in spite of passage through various experiment animals and of continuous cultivation upon dog serum through many generations.

Culture Bovine S, when first grown in pure culture, appeared as typically bovine so far as morphology is concerned. (Pl. III, fig. 1.) It displayed deviations in virulence and in cultural qualities, however, and later investigations have shown that it will develop members of unusual length, accompanied with more or less beading, rather more readily than was the case with culture Bovine T. Examination of the thirteenth generation of the stock culture of Bovine S shows a material lengthening of the members, together with occasional beading. (Pl. III, fig. 2.)

Passage through sheep also served to elongate the bacilli, as will be seen by reference to a preparation made from the nineteen days' growth of the third generation on dog serum. (Pl. III, fig. 3.) A material extension in the length with frequent beading also follows passage through a series of cats or rabbits, as is indicated by the same aged growth of the same numbered series. (Pl. III, fig. 4.)

Culture Hog O, derived from a mesenteric lymph node of a hog, adhered closely to the bovine type when first recovered, and continued cultivation for three years upon dog serum has caused no apparent changes. As with some of the other cultures, passage of culture Hog O through a series of cats served to give a permanent extension of length (Pl. III, fig. 5), and the effect of passage through the rabbit proved to be very similar. (Pl. III, fig. 6.)

There was never any great difference observable between culture Hog O and culture Sheep M, the latter originating from the mesenteric lymph gland of a sheep. The effects of long-continued cultivation and of animal passage were very similar upon these two cultures. Plate II, figure 4, shows the original sheep culture after it had begun to make noticeable growth, and figure 5 represents this strain of bacilli recovered from the seventh of a series of rabbits after growth for two generations upon dog serum, while figure 6 of the same plate shows the effects of passage through a series of six cats followed by cultivation for four generations upon dog serum.

REVIEW OF MORPHOLOGICAL RESULTS.

It is interesting to review the results obtained by this study of the morphology of the above cultures on dog serum. We find that a marked difference is shown between bacilli of human origin and also between those organisms obtained from sheep, hog, and bovine. Too great dependence, therefore, should not be placed upon the morphology of organisms alone in determining their origin, although it is now quite generally conceded that marked differences are frequently shown to exist between tubercle bacilli derived from different sources. In recognition of this repeatedly demonstrated fact it has become customary to refer to short, straight, stubby bacilli, which show no beading when stained by the usual methods, as being representatives of the bovine type. Those that appear in various lengths, irregularly curved and beaded, and when grown upon solid media develop in dry, profuse colonies more or less granular, are referred to as being of the human type, while those of somewhat similar conformation to the latter, although longer, that produce a smooth, moist, amorphous growth upon solid media, are considered avian in type. Many cultures will be met, however, which, if judged by the above standards alone, will prove very puzzling to the investigator, and consideration of the species of the host which produced them, together with their cultural characteristics and their virulence toward experiment animals, will not give a positive solution to the problem. Some writers base their classification of tubercle bacilli upon morphology alone and are at once confronted by bovine and human types in a single human cadaver or by a similar combination and various mixtures with the avian type in one of the domestic animals. They explain such occurrences as these by arguing that they are dealing with a mixed infection, and that the patient actually harbors two or more species of tubercle bacilli at one time.

In the course of the investigations here recorded the writers have examined several thousand microscopic preparations from tubercle cultures and feel impelled to state in the light of these numerous presentations that a commingling of different types of tubercle bacilli in a single subject is by no means of rare occurrence if one is to judge by morphology alone. Furthermore, we have noted in this study that cultures Boy V and BB correspond in every particular to the types spoken of as bovine, yet both were derived from children; while probably the longest bacilli we have recovered from tissues came from the monkey culture and a culture (undescribed in this paper) derived from the tubercular vertebra of a cow. If we adhere strictly to the classification of these bacilli by their conformation, it is necessary to admit that cultures Boy V and BB are bovine organisms infecting human beings, and that the monkey culture and the culture from the vertebral abscess of a cow were human organisms infecting these animals, but that their sojourn in their respective new hosts was not of sufficient length for them to develop those qualities induced by their environment.

It seems perfectly plausible to accept the theory that the effect of the tissues of the human body on any tubercle organism is gradually to elongate it and render it thin and beaded, while the same organism in cattle, owing to its peculiar environment, is shortened and made more solid and stubby. An analogous condition has been proved by various writers in their experiments with tubercle bacilli on culture media. Surely if it be accepted that the tubercle bacillus on egg media is made short, stubby, and solid, and the same organism in glycerin-bouillon becomes elongated and beaded, it can be argued by analogy that the body of cattle is a culture medium which shortens the germ, while the living culture medium furnished by the body of man serves to elongate the germ, as does glycerin-bouillon. Therefore, while we agree that the differences which exist between many human tubercle bacilli and the majority of bovine bacilli are sufficient, when these extremes are met, to classify them tentatively as types or varieties, we must adhere to the belief that both germs originate phylogenetically from a parent stock, and that the differences assumed in the bodies of man and cattle are the result of environment that can be approached by inoculating either germ into various appropriate culture media. Natural infections of cattle, hogs, sheep, horses, cats, fowls, and man have been reported by various authors in which the bacillus has been found to be identical in every respect with those classed as bovine. Here it will be seen that the natural distribution of bovine tubercle bacilli covers a very wide range, including, as it does, ruminants, omnivora, solipeds, carnivora, and fowls. Bacilli of the type recognized as human have been found attacking a still wider range of subjects, while bacilli of the avian type have been isolated from horses, cattle, rats, mice, man, and very many species of birds.

The more this subject is studied the more numerous are the instances noted in which the bacilli appear spontaneously in animals that are far removed from the species supposed to be their natural host, thus proving that the fields for their natural infection are not bounded by any hard and fast lines and that there is no character possessed by any tubercle bacilli in one host that may not be found in tubercle bacilli in another host, under favorable conditions. Is it not therefore reasonable to consider these various types of tubercle bacilli obtained from these different hosts as belonging to the same species of organism and showing in alteration of form the effect of sojourn in the bodies of different animals? May it not also be assumed in those cases where various types of tubercle bacilli are obtained from one animal that these are transitional forms which have not had time or opportunity during their sojourn to become transformed into the usual type peculiar to this host? It is not an uncommon experience in laboratory work to find that certain characteristics are lost by repeated cultivations, and by the continuation of such conditions certain characteristics become prominent which did not obtain in the original culture. Such organisms may be returned to their original characteristics by injecting them into animals, and it is therefore generally stated that these changes are due to unfavorable environment.

A more complete discussion on this subject will be given in another paper on the transformation of tubercle bacilli.

PATHOGENESIS.

VIRULENCE FOR RABBITS.

In the inoculation of rabbits for purposes of comparative study the subcutaneous method was adopted in all cases except with a series of five rabbits used in the work with culture Girl I. The inoculations were made on the left side of the animal, at a point 20 mm. posterior to the supero-posterior angle of the scapula. The amount of material injected was 0.5 c. c. in practically every instance, and careful endeavor was made to have this quantity represent approximately a similar number of bacilli in all cases. All cultures had been recently isolated and were injected in most cases when between 20 and 34 days old, usually of the first or second generation, but occasionally of the third generation. The animals were not disturbed after their inoculation, no attempt being made to take their temperatures.

The results have been collected in tabular form for purposes of ready comparison. (See p. 44.) It will be seen that the first rabbit of each series was inoculated with the pure culture and that each succeeding rabbit was injected with an emulsion in physiologic saline solution of some tubercular tissue from the preceding animal. In preparing the material for the original inoculations great care was used to comply with the conditions outlined by Theobald Smith in regard to the number of bacilli to be introduced at one inoculation.

For this purpose the standard of a 24-hour-old bouillon culture of typhoid bacilli was used in the determination of the density of the suspension of tubercle bacilli injected.[a]

The initial weights of the rabbits used ranged from 1,320 to 1,660 grams in most instances. In very exceptional cases it was impossible to obtain rabbits of this size and heavier ones were inoculated.

RESULTS OF RABBIT INOCULATIONS.

In making the initial inoculation with culture Girl I, the material used was the second subculture on dog serum and was of twenty days' growth. The culture was at this time showing a vigorous growth, and the individual bacilli were therefore of necessity in a thriving condition.

From the fact that this first rabbit of the series lived for one hundred and seventy-seven days one must conclude that the Girl I culture is far removed from the bovine type. The lesions developed, however, were scattered throughout the visceral organs and lymphatic glands of both the thoracic and abdominal cavities. Post-mortem examination disclosed at the point of inoculation a subcutaneous abscess about 2 cm. in diameter in the center of which was a small amount of creamy pus.

Rabbit No. 574, second in the series, was inoculated with an emulsion of one of the tuberculous lymph glands of the preceding animal. The advance of the disease was more rapid in this case, and, in addition to the tuberculous ulcer left at the point of inoculation by the rupture of the walls of the inoculation abscess, there was a surrounding zone, some 16 mm. broad, in which the subcutaneous tissues were permeated with small tubercular foci more or less confluent. In this case also the visceral organs of both of the body cavities were very generally involved.

The next rabbit of the series evidently died from the interference of respiration which necessarily followed the serious occlusion of the air spaces of the lungs by the enlargement of the tubercular growths within this organ, as the lesions in other parts of the body were not as far advanced as in the preceding animal, and were not apparently sufficient to have been the cause of death. At the point of inoculation a firm tumor developed, but, instead of degenerating and forming a circumscribed abscess, the growth was dense and fibrous. The lesions of the axillary lymph glands were dry and firm, with no remaining evidences of inflammatory extension. Kidneys and spleen were just beginning to evidence recent attack.

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[a] We were very fortunate in the fact that we were able to leave this work to Dr. J. R. Stewart, who had previously been associated for many years with Smith in his research work and who had thereby become thoroughly versed in his methods. We wish to express our obligations to Doctor Stewart for his valuable assistance rendered in this and many other portions of the work.

The inoculation of rabbit No. 824 resulted in a disease of very slow development. At the post-mortem examination, however, it was found that although the animal had increased in weight during the development of the disease, there were but few of the visceral organs or glands that had failed to suffer from attacks of the invading micro-organisms. At the seat of the injection was a firm, dry tumor which showed no tendency toward softening. The costal pleura had a covering of inflammatory tubercular neoformations over a wide area in the regions near the sternum. Lungs, liver, spleen, and kidneys were seriously involved. The average length of life of the above animals after injection was one hundred and eighteen and three-quarters days.

The later rabbits of this series were inoculated intravenously, and further description of them may therefore be dispensed with, as they can not be used in comparison with the rabbits of the other series.

Turning to culture Boy V, we find that in spite of the greatest possible care to make the inoculation similar throughout there was a considerable variation in the results obtained. Rabbit No. 646, receiving the pure culture from dog serum, second subculture of twenty-one days' growth, developed a widespread infection of tuberculosis, which on post-mortem examination was seen to consist of an infinite number of minute foci thickly sown about in the substance of the lungs, liver, spleen, and kidneys, while the various lymph glands of the body were similarly infected, and the intestinal walls in the ileocecal region were thickly studded with small whitish nodules.

Although the period of survival varied from twenty-four to eighty-four days with the rabbits of this series, the picture presented by the post-mortem examination of each one (except the fourth—No. 832—which seemed slightly milder than the others) was that of a virulent, rapidly advancing type of tuberculosis, which readily assumed inflammatory properties, as was evidenced by the presence of a zone of inflammation, more or less broad, surrounding many of the tubercular foci in the various organs. The average period of life of the rabbits of this series was fifty-nine days, which indicates a greater degree of virulence than was possessed by culture Bovine S, in which the average duration of life with the inoculated rabbits reached sixty-seven and five-sixths days.

The material used in testing culture BB upon rabbits was a culture from a tubercular nodule removed from the mesentery of cat No. 43, which was the fifth of its series, as will be seen by referring to the tabular report upon cats on page 51. This variation from the customary method of procedure was deemed justifiable because of the fact that BB had already been very thoroughly studied by Ravenel in regard to its action upon rabbits. Rabbit No. 804, which served as the first of the series, lived for sixty-two days following the inoculation, and at autopsy presented lesions very similar to those developed in rabbits by the use of culture Boy V, except that there was a more general extension of tubercular processes to the pleural and peritoneal membranes. This tendency was shown throughout the entire series. The average duration of life with the rabbits of this series was sixty and one-half days, and at autopsy each animal disclosed tuberculosis of a severe and generalized character.

The effect of inoculating culture Sputum C upon rabbits was more surprising. If we discard No. 634 from the calculation because of an accident which necessitated its destruction on the twenty-first day, and No. 741, which was chloroformed on the thirty-sixth day in order to furnish material on that particular date for a comparative test, we find that the average duration of life with this series was fifty-three and one-fifth days. This attests the remarkable pathogenicity of this sample of sputum. The first two rabbits of the series were inoculated with the third subculture from dog serum after growing for twenty-three days upon this medium. At this time the bacilli were noticeably short for sputum bacilli, and there was also an unusual proportion that stained evenly, showing no beading. At the autopsy of No. 521, on the seventy-fourth day, it was shown that the inoculation had produced an open ulcer at the seat of the inoculation. The axillary lymph glands of both right and left sides were badly tuberculous. The thyroid, sublingual, prepectoral, mediastinal, mesenteric, and knee-fold glands all contained tubercular foci, some showing many while others presented only two or three foci. The lungs were very much enlarged, and all lobes showed miliary tuberculosis, being partially made up of a confluent mass of tuberculous foci. The kidneys both contained many tuberculous foci, some of which were quite large and extended above the surface of the renal tissue. The bladder contained a quantity of urine in which considerable flocculent precipitate was present.

Rabbit No. 559, second of the series, was inoculated with an emulsion of a tuberculous axillary gland from the preceding animal and succumbed in thirty-three days. The lesions shown by this animal were very similar in extent and character to those seen in its predecessor. The rapidly fatal termination can not be traceable to the fact that the material used in the inoculation was taken directly from animal tissues instead of from a culture tube, for we find rabbit No. 794, the eighth of the series, surviving for seventy-six days under identical conditions. It appears to be an instance where individual susceptibility played a prominent part. Examining the course of the disease throughout the entire series, we find that it was in each instance acute and rapid. The bacilli quickly found their way from the point of inoculation to the internal organs and began immediately to exert their destructive influence wherever a foothold was gained. Several of the animals developed tubercular growths upon the pleural surfaces. These were usually flat with broad bases, showing no tendency toward stem formation. An extension of the growth over a longer period of time might have altered the results in this respect.

In the work with culture Case XIV, human, it was found that pure cultures after artificial cultivation were almost without effect upon rabbits. The first rabbit used was chloroformed forty-eight days after inoculation, but showed not the slightest evidence of tubercular infection. A similar result followed the inoculation of a second rabbit, as was shown by an autopsy held fifty-seven days afterwards. The third attempt resulted in the formation of a small degenerating tumor at the seat of inoculation, slight hemorrhagic swelling of the axillary glands, and the formation of numerous small foci in the lungs. An attempt to perpetuate the organisms collected from these lesions by injecting them into a fourth rabbit proved to be a complete failure, as no tubercular alterations resulted.

All inoculations of rabbits with this culture were now suspended for some time, and it was only when an accentuation of virulence had been obtained by passage through a series of dogs that this part of the experiment was resumed. When an autopsy was held upon dog No. 82, the third canine in the Case XIV series, a generalized case of tuberculosis was found, and two rabbits were immediately inoculated each with 1 c. c. subcutem of an emulsion of a bronchial lymph gland prepared in physiologic salt solution. Both of these rabbits soon gave evidence of serious infection, and both developed generalized cases of tuberculosis. No. 884 served as the first of the new series of rabbits. Although living but forty-six days after the inoculation, it gained 56 grams in weight during that time. Nevertheless the lesions were found to be widely scattered when the autopsy was held. At the seat of inoculation a shallow wide-spreading tumor, formed of a collection of various sized caseous nodules, had formed. The axillary glands were enlarged, hemorrhagic, and contained many caseous foci. The thymus glands showed similar changes. Bronchial and mediastinal glands were likewise altered. The lungs were crowded with large, round tuberculous growths, of which those near the surface projected prominently. The kidneys were thickly studded with miliary foci, while the spleen was enlarged, inflamed, and mottled. Lymph glands of the mesentery showed enlargement, and numerous caseous foci were present.

With the degree of pathogenicity now acquired by Case XIV for rabbits, no trouble was experienced in extending the test through the entire series, with an average duration of life of forty-eight and two-thirds days after the inoculation. The disease assumed a virulent, progressive character in each instance, thus illustrating the possible transformation which may be brought about in tubercle bacilli by passage through animals of a species different from their original host.

In the monkey culture (also the eight sputum cultures, and the two from urine which were classed with it) a type of tubercle bacilli was encountered which possessed only the slightest degree of virulence for rabbits. By the time that this culture had been passed through three rabbits with negative results it had proved to be without pathogenicity for larger test animals of other species, and consequently all further experiments upon rabbits were dropped. Moreover, the attempt which was made to increase its virulence, as was done with culture Case XIV, also resulted negatively.

Every inoculation of rabbits with tubercle bacilli of culture Bovine S resulted in tuberculosis of a generalized character. While its virulence for rabbits was more nearly equal to that of the other bovine as well as the porcine and ovine cultures used than was found to be the case with other species of test animals, there was still a noticeable tendency to form lesions that were lacking in inflammatory character. The slightly greater duration of life after the inoculations is not marked enough to be worthy of comment if conclusions were to be drawn from the rabbit tests alone, but may be taken into consideration in the final estimate of the pathogenicity of this culture. The material used in inoculating the first rabbit of the series was the second subculture of twenty-four days' growth. At the autopsy, seventy-seven days later, the lungs and spleen were found to be apparently unaffected. The axillary glands and the lymph glands of the thoracic and abdominal cavities contained no visible tubercular change. At the seat of the inoculation a circumscribed tuberculous tumor had formed which was of a soft, caseous nature within the surrounding capsule. The costal pleura showed several areas of fibrous inflammation. One-third of the omentum was closely covered with fibrinous neoformations, the more mature of which appeared like small " pearly " bodies. Kidneys and liver contained many miliary foci. In the case of the second rabbit of the series the lungs quickly became involved, and the death of the animal was thereby hastened. The remaining members of the series returned closely to the conditions of the first rabbit, both as to duration of life and as to the character of the lesions produced.

Culture Hog O proved to be very virulent for rabbits. Excluding the second of the series, which met with an accident, the duration of life with the series averaged sixty and five-sixths days. In the post-mortem examination of each member of the series a rapidly advancing and widely diffused type of disease was shown.

Culture Sheep M produced lesions very similar to those following the use of culture Hog O. The material used at the commencement of the series was the second subculture of twenty-three days' growth. The lesions developed consisted of a degenerating tumor at the seat of inoculation, with numerous minute foci in lungs, spleen, kidneys, liver, and lymphatic glands, which showed how thoroughly the work of carrying the bacilli to the distant tissues had been performed. The average duration of life after injection of these animals was sixty-six and three-sevenths days.

SUMMARY OF RABBIT INOCULATIONS.

If now we endeavor to inspect critically the above results it must appear evident that the individual resistance manifested by these rabbits varied to a great extent. However, the number of animals in each series serves to reduce this influence upon the results to a minimum. In comparing the degrees of pathogenesis of the various cultures used, one of the most striking features is to find the unusual virulence of Sputum C for rabbits, a virulence which was not established with any of the other experiment animals tested. Cultures Boy V and BB are also seen to possess pathogenic powers equal if not superior to the cultures of bovine type. Thus if comparison be made between the first series of inoculations with pure cultures of the various bacilli, or if the entire series in each case be averaged, the conclusion is evident that these three human cultures possess for rabbits by subcutaneous inoculation a virulence greater than is possessed by culture Bovine S, and similar to that shown by cultures Hog O and Sheep M. On the other hand culture Girl I possessed only slight virulence for rabbits, while the original culture of Case XIV and the monkey culture were practically without effect on these animals.

Table showing results of inoculations of rabbits.

[G denotes generalized tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of rabbit.	Material used for inoculation.	Days alive.	Change in weight (+gain; −loss).	Result.	Remarks.
				Grams.		
Girl I	369	Culture	177	−377	G.	Disease widely generalized.
	574	Lesion from 369	67	−368	G.	Open ulcer at seat of inoculation.
	748	Lesion from 574	58	−679	G.	Very complete involvement of the lungs.
	824	Lesion from 748	173	+255	G.	Case of slow progression but of widespread infection.
	1040	Lesion from 824	18	−509	G.	Intravenous inoculation.
	1043	Lesion from 1040	18	−453	G.	Do.
	973	Lesion from 1043	35	−594	G.	Do.
	1100	Lesion from 973	20	−396	G.	Do.
	1055	Lesion from 1100	21	−113	G.	Do.
Boy V	646	Culture	64	−453	G.	Lesions consist of numerous small tubercular foci, widely disseminated.
	707	Lesion from 646	46	−30	G.	Every indication of rapid inflammatory invasion.

Table showing results of inoculations of rabbits—Continued.

Source of bacilli.	No. of rabbit.	Material used for inoculation.	Days alive.	Change in weight (+gain; −loss). Grams.	Result.	Remarks.
Boy V	759	Lesion from 707	55	+ 226	G.	Lungs and pleuræ most seriously affected.
	832	Lesion from 759	81	− 226	G.	Many tubercular fibrous growths attached to parietal pleuræ.
	901	Lesion from 832	24	− 340	G.	Inflammatory form of disease.
	934	Lesion from 901	84	− 566	G.	Disease widely generalized.
B B	804	Culture from cat 43.	62	+ 56	G.	Severe type of disease.
	847	Lesion from 804	79	− 170	G.	Whitish pearly growths upon costal pleuræ.
	935	Lesion from 847	46	+ 226	G.	Pearly growths upon costal and mediastinal pleuræ.
	926	Lesion from 935	61	− 308	G.	Pearly growths upon costal and diaphragmatic pleuræ.
	1025	Lesion from 926	61	− 566	G.	Severe type of disease.
	1077	Lesion from 1025	54	− 538	G.	Generalized and extensive lesions.
Sputum C	420	Culture	59	− 62	G.	Advancing inflammatory type.
	521	Culture	74	− 680	G.	Severe progressive lesions.
	559	Lesion from 521	33	− 84	G.	Severe type.
	575	Lesion from 559	50	− 280	G.	Rapidly advancing disease.
	634	Lesion from 575	21	....	L.	Leg broken.
	650	Lesion from 634	58	− 340	G.	Extensive diffused tuberculosis. Lungs firmly adherent to costal walls.
	723	Lesion from 650	35	− 56	G.	Moderate type.
	741	Lesion from 723	36	− 112	L.	Chloroformed to gain material for comparative test.
	794	Lesion from 741	76	− 30	G.	Widely generalized case.
	858	Lesion from 794	42	− 210	G.	Costal and diaphragmatic pleuræ show numerous white tubercular growths.
	680	Lesion from 858	55	− 906	G.	Pericardium and costal and mediastinal pleuræ sown with small white tubercular growths.
	962	Lesion from 680	50	− 566	G.	Lesions widely generalized.
Case XIV	853	Culture	48	+ 30	N.	Chloroformed.
	844	Culture	57	+ 56	N.	Do.
	654	Culture	105	+ 170	L.	Chloroformed; very mild form of disease.
	762	Lesion from 654	79	+ 283	N.	Chloroformed.
	834	Culture from dog 82.	28	− 226	G.	Chloroformed; progressive spreading case.
	884	Culture from dog 82.	46	+ 56	G.	Progressive spreading case.
	964	Lesion from 884	59	− 255	G.	Inflammatory type.
	783	Lesion from 964	59	− 509	G.	Widely generalized case.
	894	Lesion from 783	57	− 877	G.	Strong growth of pearly bodies upon costal and diaphragmatic pleuræ.
	1134	Lesion from 894	37	− 113	G.	Most serious lesions are in lungs.
	1084	Lesion from 1134	34	− 150	G.	Lesions widely generalized.
Monkey	373	Culture	40	0	L.	Only lesion is at point of inoculation.
	418	Lesion from 373	247	−1,472	N.	Death resulted from intercurrent disease. Very large rabbit.
	699	Culture	118	+ 113	N.	Chloroformed.
Bovine S.	375	Culture	69	− 450	G.	
	697	Culture	77	− 56	G.	Moderately invasive type.
	567	Lesion from 697	50	− 425	G.	Widely generalized case.
	764	Lesion from 567	65	− 340	G.	Moderately invasive type.
	835	Lesion from 764	74	− 453	G.	Costal and diaphragmatic pleuræ largely covered with small, flat, whitish tubercular tumors.
	846	Lesion from 835	72	− 538	G.	Mild type of disease.
Hog O	539	Culture	57	− 340	G.	Widely diffused case.
	576	Lesion from 539	12	− 113	L.	Chloroformed; leg broken.
	577	Lesion from 576	48	− 340	G.	Rapidly advancing type.
	633	Lesion from 577	42	− 906	G.	Do.
	675	Lesion from 633	69	− 652	G.	Costal and diaphragmatic pleuræ studded with small tubercular nodules.
	747	Lesion from 675	58	− 30	G.	Costal pleura and pericardium support very numerous pearly growths.
	815	Lesion from 747	91	− 624	G.	Costal pleura supports numerous tubercular growths.
Sheep M	441	Culture	96	− 680	G.	
	360	Lesion from 441	63	− 283	G.	Severe type.
	602	Lesion from 360	58	− 283	G.	Do.
	676	Lesion from 602	47	− 210	G.	Do.
	722	Lesion from 676	58	− 226	G.	Shows extensive tubercular infection of the intestinal walls.
	788	Lesion from 722	52	− 226	G.	Very general infection.
	838	Lesion from 788	91	− 396	G.	Do.

VIRULENCE FOR WHITE MICE.

A number of inoculations of white mice were made with tubercle bacilli from each culture. In most cases the results were entirely negative. In others, while there were no microscopic tubercular lesions, there would be noted a considerable enlargement of the kidneys. Microscopic examination of smears taken from kidneys in this condition usually disclosed the presence of numerous scattered bacilli.

So few of the mice developed tubercular lesions, and those which did develop them had so little relationship one with another, that the presenting of the results in tabulated form seems to be sufficient for the purposes of this investigation. It may be stated, however, that the inoculations were uniformly made subcutaneously at the base of the tail.

Table showing results of inoculations of white mice.

[G denotes generalized tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of mouse.	Material used for inoculation.	Size of dose.	Days alive.	Result.	Remarks.
			c. c.			
Girl I	3	Culture	0.25	87	N.	
	8	Culture	.15	119	N.	Chloroformed.
	22	Culture	.10	95	N.	Do.
	42	Culture	.10	127	N.	Do.
Boy V	9	Culture	.15	8	N.	Death due to pneumonia.
	12	Culture	.25	78	G.	Axillary glands and kidneys contained tubercle bacilli.
	25	Lesion from 12	.10	19	G.	Lungs and kidneys contained tubercle bacilli.
	26	Lesion from 25	.10	7	N.	
	27	Lesion from 25	.10	17	N.	
	28	Culture	.30	61	L.	Kidneys contained a few tubercle bacilli.
	34	Lesion from 28	.10	73	N.	Chloroformed.
BB	4	Culture	.25	196	L.	Chloroformed; lungs filled with tubercular nodules.
	17	Lesion from 4	1.00	122	N.	Chloroformed; no tubercle bacilli could be recovered.
	31	Culture	1.00	26	L.	Tubercle bacilli were found in the kidneys.
	33	Lesion from 31	.20	3	N.	Death due to septicemia.
	37	Lesion from cat 54	.20	4	N.	Death caused by intercurrent disease.
	38	do	.20	4	N	Do.
Sputum C	1	Culture		196	L.	Tubercular lesions present in lungs.
	16	Lesion from 1	1.00	122	N	Chloroformed; apparently free from tuberculosis.
	32	Culture	1.50	17	N.	
	35	Lesion from rabbit 794.	1.00	16	G.	Lungs, liver and spleen support many tubercular foci.
	48	Lesion from 35	.20	25	N.	
	43	Lesion from rabbit 858.	.10	26	G.	Precrural glands, liver, and spleen support tubercular foci.
	45	Lesion from 43	.10	2	N.	Death due to intercurrent disease.
Case XIV	10	Culture	.15	2	N.	Death caused by pneumonia.
	14	Culture	.25	12	G.	Lungs and spleen contain tubercular foci.
	20	Lesion from 14	.10	53	N.	
	23	Culture	.10	56	N.	
	29	Culture	.10	78	G.	Lungs and precrural glands contain caseous foci.
	44	Lung of dog 82	.10	15	N.	
	46	Culture	.10	11	G.	Spleen and precrural glands tubercular.
Monkey	2	Culture	.25	196	N.	Chloroformed.
	19	Culture	1.00	53	N.	
Bovine S	8	Culture	.10	53	G.	Spleen and kidneys swarming with tubercle bacilli.
	21	Lesion from 8	.10	94	N.	
	40	Culture	.10	69	N.	
Hog O	5	Culture	.25	10	G.	Point of inoculation and right kidney positive.

Table showing results of inoculations of white mice—Continued.

Source of bacilli.	No. of mouse.	Material used for inoculation.	Size of dose.	Days alive.	Result.	Remarks.
			c. c.			
Hog O	15	Culture	0.25	103	N.	
	30	Culture	.10	41	N.	
	41	Culture	.10	127	L.	Chloroformed; one small healed lesion in lungs.
Sheep M	6	Culture	.20	25	L.	Death due to pneumonia; tubercle bacilli recovered from seat of inoculation.
	7	Lesion from 6	.20	94	N.	
	13	Culture	.20	69	G.	Chloroformed; tubercle bacilli recovered from lungs, spleen, and kidneys.
	24	Lesion from 13	.10	67	N.	
	39	Culture	.10	69	L.	Tubercular foci located in lungs.
	47	Lesion from 39	.10	11	N.	

VIRULENCE FOR CATS.

In the experimental work with cats a change was made in the manner of making inoculations, intrathoracic injections being adopted instead of the subcutaneous method used upon the test animals of other species. A dosage of 1 c. c. was uniformly employed, and the usual gauge of density was followed in the preparation of the material from dog-serum cultures which served for the initial inoculations. The custom was followed, as in all the other experiments, of injecting the first animal of each series with a young culture of bacilli grown on solidified dog serum for one, two, or three generations, while the others of the series received the same sized dose of an emulsion of a tubercular gland or other suitable tissue of each preceding animal. When such lesions were not produced recourse was had to the bacilli in culture for the purpose of injecting in continuation of the series.

RESULTS OF CAT INOCULATIONS.

The results from the inoculation of bacilli of culture Girl I bordered so closely upon failure in each instance that no serial tests could be made. Reference to the table on page 51 will show that none but localized lesions were developed, and that these were inclined to heal without affecting the general health of the cat or causing it to lose in weight.

The next culture, isolated from Boy V, presented a marked contrast in the matter of pathogenicity. The first cat—No. 26—to be inoculated with this culture received bacilli from a second subculture on dog serum after thirty days' growth on this medium. The animal quickly began to give evidences of serious illness. It became drowsy and lost its desire for food. Its coat became rough and staring. A sticky fluid began to gather about the corner of the eyes, and this became more and more profuse until a day or two before death the eyes were completely closed. Respiration finally became labored and death occurred on the twenty-third day. This brief outline will serve to indicate the acute course which the disease followed in the case of cat No. 26. The autopsy showed the following conditions: Both eyes were closed with an accumulation of purulent exudate of greenish-yellow color which was teeming with tubercle bacilli. At the point of injection there had developed a small subcutaneous abscess filled with creamy pus. The axillary lymph gland on this side showed involvement. The principal lobes of the lungs contained hemorrhagic areas filled with various-sized tuberculous foci. The remaining lobes appeared much contracted, having a rough, serrated appearance caused by the pressure of the large quantity of effusion in the thorax. The mediastinal glands were markedly tuberculous. The entire pleural cavity was lined with a cream-colored, tenacious exudate. No lesions of a tubercular nature were noted within the peritoneal cavity.

The other cats in this series offered no material change in the course or character of the disease until cat No. 55, the tenth of the series, was reached. In this instance the cat showed no deleterious effects from the inoculation, but instead was evidently gaining in weight, denoting a great individual resistance to tuberculosis. It was therefore decided after one hundred and twenty-five days had passed to chloroform the animal. It was now found that the bacilli had been widely disseminated throughout the lungs, the liver, and the spleen, and that these organs were thickly studded with tubercular foci. As the greater number of these areas were miliary in character it seems probable that the secondary invasion was of rather recent occurrence, and it is even possible that the natural resistance of cat No. 55 was so great that she would have ultimately recovered had chloroform not been called into requisition. The remaining cats of the series showed no material deviation in results produced from those displayed by the leading members of the series, and no attenuation of virulence of the tubercle bacilli could be detected to which the slow invasion in case of cat No. 55 could be assigned. Taking a review of the series as a whole, it will be seen that culture Boy V is possessed of a high degree of virulence for.cats.

Culture BB was less active than the preceding, but produced tuberculosis of a generalized type in each cat inoculated. The first cat of this series was inoculated with a suspension of pure culture of twenty days' growth, which was the twelfth generation since the isolation of the micro-organism. At the post-mortem examination, held one hundred and fifty-four days later, a large, subcutaneous, firm tumor was found at the point of inoculation. The lungs were thickly sown with small foci, but without inflammatory infiltration. The liver and spleen were seriously affected and were enlarged to fully double the normal size. Cat No. 9, second of the series, showed lesions very similar to the above, but did not survive the inoculation for as long a period. A gain in weight was shown by cat No. 48, the sixth of the series, but all the others in the test showed a decrease.

Considerable variation was shown in the effects of bacilli of culture Sputum C upon the several cats of the series. At the beginning of the series only localized lesions were produced, but increase of virulence was apparently gained by passage from animal to animal. The material used at the initial inoculation had grown for twenty-five days upon dog serum and was of the fourth transfer. On chloroforming cat No. 6, one hundred and thirty-eight days after the inoculation, the only lesion to be found consisted of a tubercular growth some 4 mm. in diameter located in the anterior lobe of the left lung. From this nodule a number of tubercle bacilli could be demonstrated microscopically, and the substance of this lesion served as the material for the transfer of the culture to the second cat of the series, when similar results were obtained. The third cat of the series was pregnant, and the depletion caused by parturition apparently broke down her natural resistance, as the tubercle bacilli gained access to the organs and glands of the abdominal cavity, where they produced countless virulent foci. The disease seems to have gained an access of virulence from this point, and to have later acquired an inflammatory character which persisted until the ninth cat of the series was reached.

Pure culture of Case XIV, removed from dog serum twenty-four days after second transplantation, served for the initial inoculation. Cat No. 27 received this material and was chloroformed seventy-six days later. The lungs and costal pleura supported a few tubercular growths, but these were of feeble character and did not serve to lessen the vitality of the host. Transferred to the next cat of the series no more extensive lesions were produced, and passage to the third brought negative results. From the slight lesions resulting from the inoculation of cultures of Case XIV into cats it was decided to begin a new series, using an emulsion from dog No. 82, as considerable pathogenicity had been shown by this culture for dogs, and from the lesions developed in dog No. 82 bacilli could be obtained that were active and virulent. The results give evidence of the enhancement of virulence attained by Case XIV by passage through dogs. The remaining cats of the series developed rapidly progressing cases of tuberculosis, and there was marked loss of weight and early death in each instance.

The monkey culture proved innocuous to cats, as the post-mortem examination showed no trace of tubercular lesions.

The animals inoculated with culture Bovine S curiously show a gain in weight with the exception of cat No. 40, second of the series. Although the first and second animals developed extensive lesions of tuberculosis, there was apparently a later loss of virulence, as the alterations were confined almost exclusively to the region of inoculation, and the last two cats in the series had to be chloroformed for necropsy.

Culture Hog O, which has been found to be bovine in every respect, was extremely virulent for cats from the initial test. Generalized tuberculosis of rapid invasion succeeded the inoculation of cat No. 7 with the second generation of a pure culture 27 days old from dog serum. Although the cat survived but thirty-nine days, this interval sufficed for the spread of the disease throughout the tissues of the lungs and into the peritoneal cavity, where the spleen, the liver, and the gastric lymph glands were attacked. In addition to the infection of these visceral organs, the lymph glands of the neck quite uniformly contained caseous foci, and the axillary glands were much swollen and supported numerous caseous centers. The remaining cats were each inoculated with 1 c. c. of an emulsion prepared from a tubercular gland of the preceding animal, and this was followed by marked loss of weight and an acute, generalized form of the disease in each of the members of the series. The average duration of life with the cats of this test was twenty-four and one-fifth days.

The average length of life with the cats of the series of culture Sheep M was but slightly greater than the above, as it reached but twenty-six and one-half days, while the effect of the injection and the extent of the lesions produced were practically the same. The most noticeable point of difference between these two latter cultures was in their adaptation to artificial growth after passage through cats. Culture Hog O quickly developed a luxuriant growth, while it was only by means of long-continued coaxing that culture Sheep M could be shown to have made even the slightest advance.

SUMMARY OF CAT INOCULATIONS.

A study of the results obtained by the injection of the above animals admits of the following conclusions: Cultures Monkey and Girl I are not virulent for cats. Cultures Hog O, Sheep M, and Boy V are the most pathogenic bacilli of those tested on these animals. Bovine S culture has less virulence for cats than the last-mentioned group of bacilli, while of still lower pathogenesis are cultures Sputum C and Case XIV, in the order named. As will be observed in the accompanying table, it is possible in certain instances to increase the virulence of an organism by passage through a series of animals. Similar treatment in other cases when a different bacillus is used fails to produce this enhancement. An organism possessing a moderate degree of virulence is more capable of having such virulence increased than one possessing less pathogenesis, but when the bacillus already possesses a great degree of virulence, this virulence is not capable of being enhanced to the same extent as with less pathogenic germs. Moreover, bacilli of such low virulence as is indicated in the monkey culture are not susceptible of being exalted in their pathogenicity. Certain irregular results, as cat No. 55, which lived one hundred and twenty-five days, can only be explained by the supposition that the individual resistance possessed by the animal was developed to a far greater extent than is usually observed in that species.

Table showing results of inoculations of cats.

[G denotes generalized tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of cat.	Material used for inoculation.	Days alive.	Change in weight (+gain; −loss).	Result.	Remarks.
				Grams.		
Girl I	4	Culture	104	− 226	L.	Lungs show a few miliary foci of tuberculosis.
	5	Culture	133	+1,075	L.	Chloroformed; four tubercular foci found in lungs.
	8	Culture	89	− 340	N.	Death due to impaction.
	21	Culture	100	+ 213	N.	Chloroformed.
	37	Culture	91	+ 453	L.	Chloroformed; few scattered foci found in lungs. Healed.
	52	Culture	81	+ 285	L.	Chloroformed; one healed focus in lungs.
Boy V	26	Culture	23	− 300	G.	Severe inflammatory type.
	29	Lesion from 26	19	− 453	G.	Inflammatory type.
	32	Lesion from 29	18	− 235	G.	Generalized tuberculosis, most severe in thoracic cavity.
	33	Lesion from 32	13	− 313	G.	Generalized tuberculosis, chiefly effective in thoracic cavity.
	36	Lesion from 33	15	− 226	G.	Severe generalized tuberculosis.
	39	Lesion from 36	14	− 450	G.	Severe inflammatory infection of all thoracic organs.
	41	Lesion from 39	18	− 504	G.	Generalized case, with flocculent tubercular exudate in both body cavities.
	44	Lesion from 41	34	− 792	G.	
	51	Lesion from 44	37	− 482	G.	
	55	Lesion from 51	125	+ 423	G.	Chloroformed; lungs, liver, and spleen studded with miliary foci.
	63	Lesion from 55	27	− 538	G.	Disease widely generalized.
	67	Lesion from 63	19	−1,104	G.	Death resulted from tubercular pleurisy, exudate swarming with tubercle bacilli.
	69	Lesion from 67	19	− 962	G.	Do.
	71	Lesion from 69	12	− 340	G.	Severe infection of thoracic organs.
	74	Lesion from 71	16	−1,130	G.	Inflammatory type.
BB	3	Culture	154	− 670	G.	Lesions chiefly limited to thoracic cavity, liver, and spleen.
	9	Lesion from 3	66	− 120	G.	Lungs, liver, and spleen affected; also mesenteric, gastric, prepectoral, and mediastinal lymph glands.
	18	Lesion from 9	177	− 240	G.	Chloroformed; lesions chiefly in lungs, liver, omentum, and mesenteric glands.
	35	Lesion from 18	42	− 790	G.	Lesions located in thoracic cavity and spleen.
	43	Lesion from 35	27	− 685	G.	Great accumulation of serous fluid in body cavities; lungs, spleen, pleura, and peritoneum affected.
	48	Lesion from 43	33	+ 116	G.	Tuberculosis of lungs, pleura, pericardium, and mediastinal and suprasternal lymph glands.
	54	Lesion from 48	67	− 232	G.	Severe generalized case.
Sputum C	6	Culture	138	+ 453	L.	Chloroformed; single tubercular lesion in left lobe of lung.
	17	Lesion from 6	118	− 197	L.	Chloroformed; lesions consist of a few nodules in the lungs.
	34	Lesion from 17	75	−1,443	G.	Loss in weight partly due to normal parturition; lungs, pleura, spleen, omentum, mesentery, and thoracic lymph glands involved.
	47	Lesion from 34	92	− 10	G.	Lesions widely generalized; marked tubercular pleurisy.
	56	Lesion from 47	88	+ 396	G.	Lesions chiefly confined to lungs, mediastinal and costal pleuræ, and adjacent lymph glands.

Table showing results of inoculations of cats—Continued.

Source of bacilli.	No. of cat.	Material used for inoculation.	Days alive.	Change in weight (+gain; −loss).	Result.	Remarks.
				Grams.		
Sputum C..	61	Lesion from 56.....	25	− 225	G.	Inflammatory tuberculosis of the tissues just recorded and also of the liver.
	65	Lesion from 61.....	24	− 115	G.	Lesions widespread throughout the body.
	68	Lesion from 65.....	27	−1,274	G.	Loss of weight partly due to premature parturition; generalized disease with extensive tubercular pleurisy.
	72	Lesion from 68.....	23	− 905	G.	Lungs generally involved; liver and spleen have miliary infection.
Case XIV..	27	Culture............	76	+ 396	L.	Chloroformed; lungs and costal pleura show a few tubercular growths.
	38	Lesion from 27.....	87	+ 905	L.	Chloroformed; few slight nodules in lungs and spleen.
	53	Lesion from 38.....	59	+ 482	N.	Chloroformed.
	59	Lesion from dog 82..	43	− 283	G.	Pleura thickly covered with prominent tubercular growths, also pericardium, lungs, liver, pancreas, spleen, kidneys, and lymph glands.
	60	Lesion from 59......	19	− 226	G.	Tubercular involvement of the lungs, pleura, pericardium, spleen, liver, and lymph glands.
	62	Lesion from 60.....	23	− 396	G.	Inflammatory type of tuberculosis, affecting the lungs, pleura, pericardium, liver, thoracic and mesenteric lymph glands, mesentery, and conjunctiva.
	66	Lesion from 62......	25	−1,217	G.	Inflammatory type of tuberculosis as above.
	70	Lesion from 66......	19	− 368	G.	Lungs, liver, omentum, thoracic and mesenteric glands affected with tubercular exudate in thoracic cavity.
	73	Lesion from 70......	21	− 736	G.	Lungs, liver, spleen, and pleura severely tuberculous; purulent tubercular discharge from left eye.
Monkey	1	Culture............	222	− 85	N.	Chloroformed.
	12	Culture............	101	+ 906	N.	Do.
Bovine S....	30	Culture............	74	+ 312	G.	Axillary glands, parietal pleura, lungs, pericardium, suprasternal and mediastinal glands affected.
	40	Lesion from 30......	27	− 975	G.	Inflammatory tuberculosis of axillary, bronchial, and mediastinal glands, lungs, pleura, and pericardium.
	46	Lesion from 40......	25	+ 312	L.	Lesions confined to lungs.
	50	Lesion from 46......	171	+ 453	L.	Chloroformed; disease localized in left lung and adjoining lymph glands.
	64	Lesion from 50......	173	+ 342	L.	Chloroformed; has 1 cicatricial area 1 cm. across and a few scattered tubercular nodules in one lobe of lung.
Hog O......	7	Culture............	39	− 538	G.	Generalized tuberculosis of rapid invasion.
	14	Lesion from 7.......	23	− 340	G.	Lungs, liver, spleen, mediastinal and gastric lymph glands tubercular.
	16	Lesion from 14......	16	−1,105	G.	Severe inflammatory type, involving lungs, spleen, pleura, pericardium, and thoracic lymph glands.
	19	Lesion from 16......	19	− 785	G.	Do.
	22	Lesion from 19......	24	− 850	G.	Rapidly progressive type with tubercular pleurisy.
Sheep M....	10	Culture............	44	− 623	G.	Severe infection of thoracic lymph glands, lungs, pleura, and pericardium.
	15	Lesion from 10......	36	− 603	G.	Severe infection of lungs, liver, spleen, and lymph glands generally.
	20	Lesion from 15......	18	− 905	G.	Inflammatory tuberculosis of thoracic organs with sero-fibrinous tubercular pleurisy.
	23	Lesion from 20......	22	− 340	G.	Inflammatory type, as in preceding case.
	31	Lesion from 23......	17	....	G.	Death due to intercurrent disease; numerous tubercular lesions apparent in thoracic cavity.
	31	Lesion from 31......	22	− 680	G.	Case of wide generalization.

VIRULENCE FOR DOGS.

Experiments upon a series of dogs were made with each of the principal varieties of tubercle bacilli whose study is here recorded. The natural resistance of these animals to the action of tubercle bacilli was found to be very great, although far from uniform in different individuals. Their natural powers of resistance were so strong that the greater portion of the inoculations made upon them failed to produce any lasting lesions of tuberculosis. These negative results have been attributed to the ideal housing, surroundings, and care provided for the animals, which probably assisted them in overcoming the effects of the injection, since it is usual for dogs to show a greater susceptibility to tuberculosis than is here indicated. A site was selected for the animals at the Bureau Experiment Station on a dry, partially shaded knoll, and individual cages were built well off the ground and covered with wide-mesh wire screens. Each cage was 5 by 12 feet in size and contained a dog box for protection from the weather. That this continuous life in the open air, with excellent care and plenty of food of a good quality, was instrumental in causing the dogs in many cases to resist tubercular infection was proved by the following experiment:

Two dogs, Nos. 90 and 92, of practically the same size and weight, were inoculated simultaneously under the skin of the shoulders each with 2 c. c. of a suspension of a 58-day old culture from dog No. 88. The former was placed in a warm, poorly ventilated, and improperly lighted room, while the latter was placed in one of the above-mentioned cages in the open air at the Experiment Station. The animals were similarly fed and received equal attention. They were chloroformed forty-six days later, at which time No. 90 had lost four times as much weight as No. 92. The necropsy showed the carcass of the former to have a fistulous tract on both shoulders about 10 mm. in diameter, leading along the subcutaneous tissues for 6 to 8 cm., and surrounded by firm, fibrous tissue and tubercular growths. Both prescapular glands were enlarged and contained three or four caseous foci, about the size of peas. The lungs contained numerous translucent tubercular areas scattered through all the lobes. On the other hand, the autopsy on No. 92 showed a small superficial ulcer at each point of injection, but the prescapular lymph glands, lungs, and all other organs were without lesions. Such a striking contrast is here shown of the effects of environment, or rather of hygienic and unhygienic treatment, that we believe the conclusion is warranted that the dogs in this series of experiments were made more resistant by the character of their surroundings.

Small dogs were used in these tests in all cases. Mongrel fox terriers, beagles, black and tans, or pugs, weighing from 15 to 24 pounds, represent the type of animal selected. These were inoculated by injecting the tuberculous suspension or emulsion immediately beneath the skin on both sides of the neck, just in front of the shoulder, at the beginning of the experiment. Other methods were introduced later, as will be seen by reference to the table on page 57, and some variation in the amount injected will also be seen.

Four dogs died during the course of the test, but in only one instance—that of dog No. 83, culture Boy V—could death be assigned to the tubercular invasion. All others were chloroformed after various periods of time had elapsed.

RESULTS OF DOG INOCULATIONS.

Culture Girl I may be considered nonvirulent for dogs.

Subcutaneous inoculations of culture Boy V produced no serious lesions. By the use of intrathoracic inoculation this culture was brought to a degree of virulence which enabled it to produce generalized tuberculosis in dog No. 83. This animal received 1.4 c. c. of a saline suspension of a thirty day's growth of this culture and died sixty-seven days later. The necropsy showed the lungs to be filled with small, round, glassy foci, and the costal, diaphragmatic, and visceral pleura to support numerous yellowish growths varying in size from 0.5 to 5 mm. in diameter. The kidneys contained diffused necrotic areas in the cortical portion. The right eye was partly closed from conjunctivitis, and the purulent discharge therefrom contained numerous tubercle bacilli. An emulsion prepared from a pleura tubercle of this carcass was injected subcutaneously into dog No. 88, and ninety-two days later the animal was asphyxiated. The post-mortem disclosed two tubercular swellings the size of hickory nuts at the seats of injection. The prescapular glands were enlarged and inflamed. The lungs contained many small foci, some glassy and firm and others soft and inflammatory. The pericardium, mediastinal, and suprasternal lymph glands, liver, spleen, kidneys, omentum, and mesenteric glands all supported numerous foci of tuberculosis.

Culture BB was not sufficiently pathogenic for dogs to cause spread of the disease away from the seat of the inoculation except when used intrathoracically. Even then the lesions were few and well circumscribed. This was likewise true when 2 c. c. of an emulsion of the tubercular pericardium of cat No. 54 was injected subcutaneously.

The first three dogs inoculated with a pure culture of Sputum C failed to show any lesions of tuberculosis when chloroformed from seventy-seven to one hundred and thirty days later. The fourth dog received subcutaneously 2.4 c. c. of an emulsion of the suprasternal lymph gland of cat No. 34. This dog died twenty-one days later from an internal hemorrhage caused by a ruptured liver. On post-mortem examination the points of injection were slightly inflamed and a few foci of tuberculosis were apparent in the lungs, from which tubercle bacilli were recovered. Two cubic centimeters of an emulsion prepared from these lungs were thereupon injected subcutaneously into dog No. 76, which was chloroformed eighty days later. The only lesions apparent were a very few scattered whitish foci in the lungs, in which no tubercle bacilli could be found. Dog No. 85 received 2 c. c. of an emulsified axillary gland of rabbit No. 858 subcutaneously. After being chloroformed sixty-three days later the necropsy showed a small ulcer at the seat of injection on the left side, beneath which was a slender, soft, tubercular swelling about the size of a silver dollar. No other lesions were apparent.

Culture Case XIV was used upon a series of six dogs, and generalized tuberculosis resulted in each case. The sixth of the series, No. 97, appeared unusually resistant, as the organisms were not discovered in any. of the visceral organs. It was found by using an emulsion of tissues from dog No. 82 that increased pathogenicity had been acquired for cats and rabbits by this culture during its retention in the canine organism.

The monkey culture was without pathogenicity for dogs by subcutaneous application.

Culture Bovine S was unable to produce progressive tuberculosis even after passage through a series of cats.

Culture Hog O appeared to have slightly greater virulence for dogs when first isolated than the average culture possesses, but not sufficient to cause regularly the development of tubercular lesions. The first of the series was inoculated with bacilli removed from dog serum five weeks after transplanting. The resulting lesion was a single tubercular abscess the size of a hen's egg. On section, well-defined walls were shown inclosing a mass of tenacious, fairly translucent pus, which much resembled tuberculous sputum. In this material tubercle bacilli were identified by the use of the microscope. Passing some of this material on to the second dog—No. 57—no tubercular change was produced at the point of inoculation. The lungs at autopsy were seen to contain a few scattered, glassy foci, which were apparently not advancing. The kidneys were studded with necrotic foci ranging in size up to that of a pinhead, but contained no tubercle bacilli. No effects followed the passage of tubercle bacilli from these pulmonary lesions on to the third dog—No. 70. The fourth dog—No. 78—died fifty-two days after an intratracheal inoculation with 0.8 c. c. of a seventh subculture of twenty-eight days' growth. The lungs were found after death to support a few scattered glassy foci, which were apparently healed and within which no tubercle bacilli could be discovered by microscopic examination. The mesenteric glands were greatly enlarged and congested, but no tubercular affection could here be seen. It was evident that the death of the dog was caused by enteritis.

No deleterious effects followed the injection of dogs with bacilli from culture Sheep M, and tubercle bacilli could not be demonstrated in any of the lesions produced.

SUMMARY OF DOG INOCULATIONS.

It is of interest to note that in all these tests there has usually been manifested a marked tendency toward the encapsulation of the tubercular focus whenever the injected organisms succeeded in gaining a temporary foothold immediately after their introduction. Lesions developed in the lungs are usually isolated, small, and glassy. There was no tendency toward the formation of soft, progressive, coalescing masses, but on the contrary, every effort of nature seems to have been exerted in the confinement of the intruding organisms within the smallest possible space and in surrounding them there by an impenetrable wall.

It will be observed from the appended table that the subcutaneous inoculations of 2 c. c. of physiologic saline solution in which tubercle bacilli were suspended in the amount uniformly used gave negative results in most instances. Used in this manner cultures Girl I, BB, Sputum C, and Monkey produced no tubercular alterations. The lesions developed when Boy V, Bovine S, Sheep M, and Hog O cultures were used in the manner employed with the other varieties in the comparative tests were very slight and showed no tendency to progress toward generalized invasion.

Only one of the cultures here described has been found to possess sufficient virulence for dogs to produce positive results in each instance of its application to them. The Case XIV culture has shown peculiar virulence for members of the canine family, and as it has been passed from dog to dog its pathogenesis has been found to be constant for these animals, although its effects upon other animals have shown that it possesses but a comparatively low degree of virulence in general.

The only death produced by tubercle bacilli in this experiment was in the case of dog No. 83, injected intrathoracically with a pure culture of Boy V bacilli. The fact that other bacilli were similarly injected without causing generalized lesions, if we exclude Case XIV, indicates that while the method was more severe than the subcutaneous injections the Boy V bacilli were more virulent than those which failed to produce equal alterations by a similar injection.

Table showing results of inoculations of dogs.

[G denotes generalized tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of dog.	Material used for inoculation.	Dose.	Days alive.	Result.	Remarks.
			c. c.			
Girl I	37	Culture	2	179	N.	
	55	Culture	2	121	N.	
	72	Culture	1	114	L.	Intrathoracic inoculation; glassy foci in lungs and kidneys; no tubercle bacilli recovered.
	81	Culture	1.5	93	N.	Free from tuberculosis.
Boy V	42	Culture	2	122	L.	Tubercular lesions at seat of inoculation, and 4 small glassy foci in lungs.
	54	Lesion from 42	2	[a]5	N.	Cause of death, pneumonia.
	62	Culture	2	116	L.	Numerous glassy foci from 1 to 5 mm. in lungs; few caseous centers in mesenteric glands; no tubercle bacilli could be found in any organ.
	73	Lesion from cow 302	2	87	L.	All lesions healed; no tubercle bacilli identified in several foci in apices of lungs.
	83	Culture	1.4	[a]67	G.	Intrathoracic inoculation; death due to generalized tuberculosis.
	88	Lesion from 83	1	92	G.	Widespread infection.
BB	36	Culture	2	202	N.	
	56	Culture	2	119	N.	All organs normal.
	71	Culture	1	112	L.	Intrathoracic inoculation; lungs contained numerous tubercular foci.
	80	Lesion from 71	1.5	95	L.	Intrathoracic inoculation; few glassy foci in lungs apparently healed.
	84	Lesion from cat 54	2	107	L.	Two small encapsulated foci in lungs fully healed.
Sputum C	40	Culture	2	130	N.	
	53	Culture	2	77	N.	
	69	Culture	2	102	N.	Intra-abdominal inoculation.
	76	Lesion from cat 34	2.4	[a]21	L.	Tubercle bacilli found in a few foci in lungs.
	77	Lesion from 76	2	80	L.	A few healed foci were found in lung.
	85	Lesion from rabbit 858	2	63	L.	Small tubercular ulcer at seat of inoculation, from which cultures were recovered.
Case XIV	63	Culture	2	117	G.	Point of inoculation, prescapular glands, lungs, pleura, and liver tuberculous.
	74	Lesion from 63	2.5	83	G.	Mediastinal glands, lungs, liver, and kidneys affected.
	82	Lesion from 74	1.5	50	G.	Intrathoracic inoculation.
	86	Lesion from 82	2	76	G.	Points of inoculation, prescapular glands, spleen, lungs, and kidneys affected.
	93	Lesion from 86	2	64	G.	Points of inoculation, prescapular glands, lungs, mediastinal glands, kidneys, and spleen affected.
	97	Lesion from 93	2	221	G.	Lesions confined to seats of inoculation, prescapular glands, axillary and precrural glands and lungs.
Monkey	32	Culture	1	249	N.	
	59	Culture	3	112	N.	
Bovine S	38	Culture	2	116	N.	No trace of inoculation remaining.
	64	Culture	2	110	L.	No tubercle bacilli could be identified in the numerous small, glassy foci in the lungs.
	75	Lesion from cat 40	2	89	L.	Local lesion; no tubercle bacilli demonstrated in the few retrogressive pulmonary foci.
Hog O	43	Culture	2	112	L.	Single tubercular abscess in left axillary region.
	57	Lesion from 43	2	115	L.	Lungs contain translucent foci 1 to 2 mm. in diameter.
	70	Lesion from 57	1	104	N.	Intratracheal inoculation.
	78	Culture	0.8	[a]52	L.	Intratracheal inoculation; death due to intercurrent disease; lungs have a few small foci, which seem to be healed.
Sheep M	41	Culture	2	144	N.	No evidence of tuberculosis in any gland or organ.
	58	Culture	3	112	L.	No tubercle bacilli could be identified in the 4 or 5 glassy foci in the parenchyma of the lungs.

[a] These animals died; all others were killed.

VIRULENCE FOR GOATS.

Owing to the difficulties encountered in obtaining goats for experimental purposes, the number of inoculations made upon these animals was quite limited. However, as the goat is so similar in most respects to the sheep, the record of the work with goats will be given as a complement to the tests upon other animals. These animals were all subjected to the tuberculin test before the inoculation of tubercular cultures.

The inoculations were made by injecting physiologic salt solution, in which the usual proportion of bacilli had been suspended, subcutaneously on each side of the neck just in front of the shoulder. The amount used varied. Three of the goats received 1 c. c. on each side, while with cultures Girl I, BB, and Bovine S the amount injected was double that quantity, 2 c. c. being used upon each side.

RESULTS OF GOAT INOCULATIONS.

The material from culture Girl I was the second subculture, of twenty days' growth. Although 4 c. c. were injected, the infection did not extend past the lymph glands adjacent to the points of inoculation. At the post-mortem examination no local lesion was found where the inoculations were made, but the prescapular glands were enlarged and firm and contained numerous degenerated foci filled with material of a caseous nature.

In the test of culture Boy V the usual amount of 2 c. c. was inoculated subcutaneously. The culture used was a four and one-half weeks' growth of the second generation. The great virulence of this culture was attested by the fact that the above inoculation caused the death of the animal in thirty-seven days. When examined post-mortem the carcass was found to be much emaciated. At the point of inoculation on each side was a firm encapsulated mass some 5 cm. in diameter, composed of necrosed and infiltrated connective tissue with hemorrhagic areas throughout. The prescapular glands of either side were moderately enlarged and badly tuberculous. The lymph glands of the axillary region were all more or less involved. The lungs were filled with tubercles varying in size from a pin head to a pea. All lobes were more or less involved.

A young kid some 2 months old received 0.75 c. c. of an emulsion of a prescapular gland of sheep No. 15 (Boy V bacilli) on each side of the neck. Asphyxiated one hundred and six days later, tubercular tumors were found on each side at the points of inoculation, each containing an amount of soft, creamy pus about equal in bulk to a medium-sized bean. The prescapular glands were enlarged and contained numerous scattered, calcified areas. Many foci ranging in size from a pin head to a pea were located in the lungs. These were all gritty on section from the calcareous deposits, and tubercle bacilli were present only in limited numbers.

Four cubic centimeters of Culture BB were used in the test upon goat No. 5, the material being taken from dog serum after twenty-nine days' growth. The animal was chloroformed at the expiration of two hundred and thirty-two days. No remains of any lesion at the points of inoculation could be found. The prescapular glands were severely affected, many tubercular foci with caseo-calcareous contents being present. The principal lobe of the left lung contained at its anterior border numerous coalescent foci of tuberculosis which on section showed many centers of caseation. The remaining portion of the lobe consisted of three isolated cavities containing semifluid pus. In the most anterior one the degeneration was of a more organized nature. The walls of the vomicæ presented true abscess-like surfaces with a granulating appearance. These cavities were in direct communication with several bronchi. The caudal lobe was sparsely sprinkled with small, millet-seed foci of apparent recent origin. The posterior half of the principal lobe of the right lung contained one large caseous abscess cavity of the size of a closed fist. The contents consisted of a semifluid caseous material which likewise was in direct communication with bronchi. The walls of this cavity appeared similar to those of the cavity in the left principal lobe. The remaining part of the lobe was thinly sprinkled with small foci of tuberculosis. These small millet-seed foci were likewise scattered over the ventral and the caudal lobes. Scattered throughout the spleen tissue were small, circumscribed tubercular nodules of shot-like appearance, very gritty on section.

Fatal results followed on the ninety-fifth day after the inoculation of a goat with 2 c. c. of a suspension of bacilli from culture Sputum C, twenty-eight days old, and of the second generation. At the post-mortem examination there was noted a thickening of the subcutaneous tissues at the points of inoculation. The tumors thus formed were broad, flat, and about 2 cm. in thickness. The axillary glands were enlarged, but no tubercular foci could be detected. The lungs contained two tubercular areas, one in each principal lobe the size of a man's fist, made up of isolated and confluent tubercles from 2 to 5 mm. in diameter. Many of these foci were badly broken down, their centers showing on section a flocculent degenerative deposit. Cover-glass preparations showed under the microscope large numbers of tubercle bacilli of the human type.

The injection of 2 c. c. salt solution suspension of tubercle bacilli from the monkey culture 4½ weeks old and of the first generation gave negative results. On chloroforming the animal two hundred and forty-eight days later no trace of the inoculation could be found at the autopsy.

Culture Bovine S was used in the amount of 4 c. c., second generation, of twenty-six days' growth. The animal died of generalized tuberculosis on the fifty-fifth day. The prescapular glands were found greatly enlarged and supporting numerous caseous areas. The axillary glands were similarly affected. A chain of small lymphatic glands extending along the side of the trachea showed numerous caseous foci. The left submaxillary gland also contained a tuberculous focus about 0.5 mm. in diameter. The suprasternal, bronchial, and mediastinal glands presented small tubercular areas. The lungs were quite congested, and scattered thickly throughout the whole parenchyma and over the various surfaces were caseous nodules from 3 to 5 mm. in diameter. The parietal pleura supported four or five areas of miliary tubercular growths. The portal lymph gland contained one minute focus, but no further tubercular change within the abdominal cavity was noted.

SUMMARY OF GOAT INOCULATIONS.

In summing up the results obtained by the use of these six different cultures upon goats it will be observed that the Monkey and Girl I cultures continue to be the least pathogenic of the series, while Boy V and Bovine S cultures are the most virulent. Cultures Sputum C and BB stand as of intermediary pathogenicity, the former causing the death of a goat in ninety-five days, while the latter did not kill, but produced such an extensive involvement of the lungs that it was remarkable that the animal could continue to live with such a limited amount of lung tissue remaining unaffected. The resistance of young animals to tuberculosis is clearly shown by the inoculation of the 2-months-old kid No. 9 with an emulsion of the Boy V bacilli recovered from sheep No. 15.

Table showing results of inoculations of goats.

[G denotes generalized tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of goat.	Dose.	Days alive.	Result.	Remarks.
		c. c.			
Girl I (culture)	7	4	210	L.	Chloroformed; no lesions except in prescapular glands.
Boy V (culture)....	3	2	37	G.	Animal died showing extensive tuberculous lesions of progressive type.
Boy V (lesion from sheep 15).	9	1.5	106	G.	Young kid. Chloroformed; points of inoculation, prescapular glands, and lungs tuberculous.
BB (culture)	5	4	232	G.	Chloroformed; disease extended to near-by glands and spleen and caused vomicæ in lungs.
Sputum C (culture)	6	2	95	G.	Animal died showing tuberculosis of pulmonary type.
Monkey (culture) .	4	2	248	N.	Chloroformed.
Bovine S (culture).	8	4	55	G.	Animal died severely infected with tuberculosis.

VIRULENCE FOR SHEEP.

The material used in the experiments upon sheep was prepared and inoculated in a manner similar to that employed in the tests upon goats.

RESULTS OF SHEEP INOCULATIONS.

Culture Girl I failed to produce any lesions of tuberculosis in the sheep to which it was applied.

Culture Boy V was of sufficient virulence to produce generalized lesions in each sheep of the series. The first received bacilli of twenty-one days' growth upon dog serum, fourth generation, and showed on post-mortem examination prescapular glands that were swollen and very slightly tubercular. The lungs were closely studded with miliary tubercles, while the costal pleura of the right side of the animal showed scattered foci upon its surface. The mediastinal glands were slightly affected, and a single caseous focus was located in one of the mesenteric glands. In the second of the series—No. 104—inoculated with an emulsified lymph gland of the above animal, a more severe type of disease was developed. Tubercular tumors formed at the points of inoculation, and tubercular infection extended to the prescapular glands, where it manifested itself by the formation of several small abscesses, in which tubercle bacilli were scantily present. All lymph glands of the thoracic cavity were enlarged and supported tubercular foci. The lungs were sprinkled with tubercular foci of various sizes up to 10 mm. in diameter. (Plate IV.) In the liver a few caseous nodules were formed. A lymph gland situated at the lesser curvature of the stomach was greatly enlarged through the growth of tubercular formations. Calcareous tumors the size of a turkey's egg formed at each point of inoculation in the next sheep of the series—No. 118—which received an emulsion of a lymph gland of sheep No. 104. The prescapular glands contained numerous calcified foci. The lungs were very slightly affected with firm, glassy foci. In the mesenteric glands numerous caseous foci were developed.

Culture BB produced no extension of tuberculosis past the prescapular glands of the first sheep of the test. In the second the lungs and liver were slightly involved in addition to the lesions in the prescapular glands, while in the third of the series a more severe infection of lungs and thoracic glands followed, and the lesions in the prescapular glands contained a large proportion of calcified material.

In the test of culture Sputum C light infection of lungs and prescapular glands was at first obtained, but attempts to pass the infection to the next of the series were unsuccessful. For the third sheep of this test an emulsion of an axillary gland of rabbit No. 858 was used, with the result that a widespread infection followed. At the points of inoculation firm calcareous tumors formed to which the skin was closely attached. In the subcutaneous tissues along the inferior surface of the neck were scattered numerous spherical tubercular growths the size of millet seeds. The infection of the prescapular glands was indicated by calcified foci, each of which was surrounded by a zone of inflammation. The lungs were studded with foci ranging from 1 to 6 mm. in diameter. The bronchial and mediastinal glands were swollen and contained many areas, some caseous, others of a calcareous nature. In the abdominal cavity tubercular lesions were found in the liver, in the portal lymph gland, and in various mesenteric glands.

The first sheep inoculated with tubercle bacilli from culture Case XIV died on the tenth day from another cause. Sheep No. 101, which was thereupon inoculated with bacilli of the second generation, grown upon dog serum for twenty-seven days, developed a few cheesy foci in the submaxillary glands and a large number of them in the prescapular glands. The lungs contained numerous glassy foci and one undergoing degeneration. The intervertebral thoracic glands showed tubercular infection. The liver was affected throughout with foci varying in size from a point to a bean. The second sheep of the series—No. 62—inoculated with an emulsified lymph gland of the above animal, showed enlarged prescapular glands with roughened surfaces. The left lung was adherent to the costal walls over a circular area some 25 mm. in diameter, and in the various lobes of the lungs small foci were scantily sprinkled. Of these about one half appeared glassy and healed, while the other half were inflamed and progressive. A few softened foci were in the mediastinal glands, and there was a slight infection of liver and kidneys. An emulsion of the lung of dog No. 82 was tested upon sheep No. 114 with widespread infection as the result. The submaxillary, sublingual, prepectoral, prescapular, precrural, mediastinal, portal, and gastric lymph glands all contained caseo-calcareous foci, while many of the mesenteric glands were swollen and inflamed. The lungs were sown throughout with tubercles of all sizes up to beans, the spleen was also permeated with calcareous nodules, and the liver and kidneys gave evidence of slight infection. Scattered about at random over the mesentery were various small tubercular bodies which were firm and hard when pressed between the thumb and finger. On microscopic examination tubercle bacilli were found in limited numbers.

Judging from the extension of the lesions, and the apparent degree of virulence manifested by culture Bovine S, it may very properly be classed with Case XIV as to its virulence for sheep. The first sheep of the series, receiving 2 c. c. of a suspension of bacilli from dog serum thirty-two days old and of the second generation, developed tubercular abscesses at each point of inoculation, miliary tuberculosis of the lungs, and a slight infection of the axillary glands. Double the quantity was injected into the next sheep of the series, and a greater infection was thereby obtained. At each point of inoculation the skin was adherent to the underlying tissues over an area some 50 mm. broad. Under each of these areas was an abscess some 20 mm. across, which was full of thick, cheesy pus. The adjacent lymph glands were filled with caseous foci. The lungs contained numerous glassy foci. The diaphragmatic pleura supported many fibrous neoformations. The bronchial and mediastinal lymph glands were studded thickly with small tubercular foci. Slight infection of liver and spleen was noted. Four cubic centimeters of an emulsion of the prescapular gland of sheep No. 13 was injected into the third of the series—No. 16. A few abscesses containing cheesy pus in which a few tubercle bacilli were demonstrated formed in the prescapular glands, in the lungs, and at the points of inoculation. The infection was of a mild type. Following this a sheep—No. 117—was inoculated with 2 c. c. of an emulsion from an axillary gland of rabbit No. 764. The prescapular and axillary glands developed caseo-calcareous foci. The bronchial and mediastinal glands were enlarged and contained many calcareous foci. The lungs supported a few glassy foci. The liver contained about 12 small yellowish-white lesions, while the portal and mesenteric lymph glands were thickly sown with small caseous lesions.

SUMMARY OF SHEEP INOCULATIONS.

None of the sheep inoculated with the various cultures tested died from the inoculation. With the exception of sheep No. 99, all of the animals were prepared for the investigation of the progress of the disease by asphyxiation with either chloroform or illuminating gas. For this reason it becomes difficult to determine the relative virulence of these various bacilli for sheep, as it is necessary to conclude from the extent and character of the lesions produced rather than from the length of time that the animal survived the injection. With this in view it will be possible to class the culture Girl I as without virulence for sheep, and cultures Bovine S, Case XIV, and Boy V as the most pathogenic for these animals. Culture BB appeared to be of lower virulence than the latter, while Sputum C was slightly less pathogenic than culture BB.

It is also interesting to note in the experiments conducted with sheep and goats that the latter appeared to be much more susceptible to the invasion of tubercle bacilli than sheep. This fact seems to be well borne out by an examination of the abattoir statistics of various countries, which indicates that sheep are also more resistant to natural infection with tuberculosis than goats. This point does not seem to have been given much prominence heretofore, but should receive greater consideration in the future in speaking of the relative susceptibility of these two species of animals.

Table showing results of inoculations of sheep.

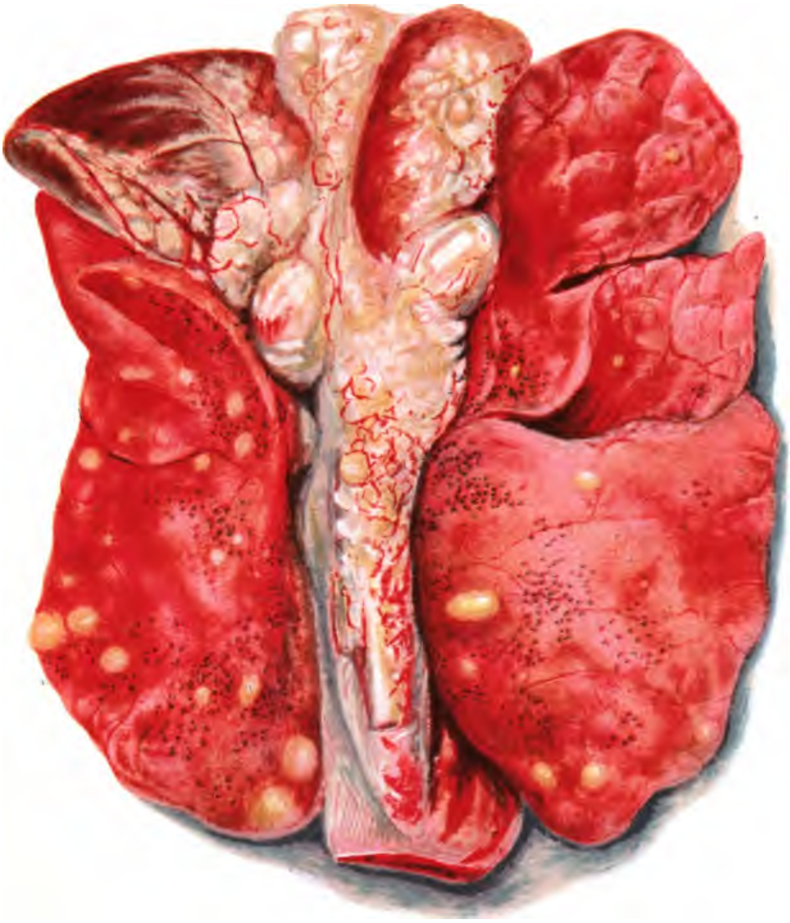
[G denotes generalized or advancing tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of sheep.	Material used for inoculation.	Dose. c. c.	Days alive.	Result.	Remarks.
Girl I	63	Culture	2	122	N.	No trace of tuberculosis found.
Boy V	15	Culture	2	44	G.	Prescapular glands, lungs, costal pleura, and mesenteric gland affected.
	104	Lesion from 15	2	94	G.	Thoracic glands, lungs, liver, and mesenteric glands affected.
	118	Lesion from 104	2	106	G.	Prescapular glands, lungs, and mesenteric glands contain tubercular foci.
BB	18	Lesion from goat 5	2	166	L.	Prescapular glands alone tuberculous.
	103	Lesion from cat 43	2	83	G.	Prescapular glands, lungs, and liver tuberculous.
	116	Lesion from 103	2	107	G.	Prescapular glands, lungs, and thoracic glands affected.
Sputum C	94	Lesion from goat 6	2	152	G.	Prescapular glands and lungs slightly affected.
	2	Lesion from 94	2	132	N.	No evidence of tuberculosis could be found.
	119	Lesion from rabbit 858	2	120	G.	Many lymph glands, lungs, and liver thickly sown with tubercular foci.
Case XIV	99	Culture	4	10	?	Died of intercurrent disease.
	101	Culture	2	101	G.	Submaxillary, prescapular, axillary, and thoracic glands, lungs, and liver tuberculous.
	62	Lesion from 101	4	89	G.	Lungs, mediastinal glands liver, and kidneys affected.
	114	Lesion from dog 82	2	119	G.	Numerous lymph glands, lungs, liver, spleen, kidneys, and mesentery tuberculous.
Bovine S	98	Culture	2	60	G.	Lungs, axillary glands, and points of inoculation involved.
	13	Lesion from 98	4	75	G.	Prescapular glands, lungs, pleura, liver, and spleen tuberculous.
	16	Lesion from 13	4	93	G.	Prescapular glands and lungs tubercular.
	117	Lesion from rabbit 764	2	102	G.	Prescapular, axillary, and mesenteric glands, lungs, and liver tuberculous.

VIRULENCE FOR CATTLE.

Greater interest attended the effects of the various cultures upon cattle than upon any other species of test animals, and the number of instances in which generalized invasion followed subcutaneous inoculation goes far to establish the fact that human tuberculosis is transmissible to cattle. No test was made with the cultures represented by culture 19, monkey, and those represented by culture Girl I proved incapable of causing any form of tubercular lesion. No test upon cattle was made with the three cultures derived from hogs, nor with the three bovine cultures represented by culture Hog O, neither with the culture Sheep S, because of the manifestly bovine character of them all.

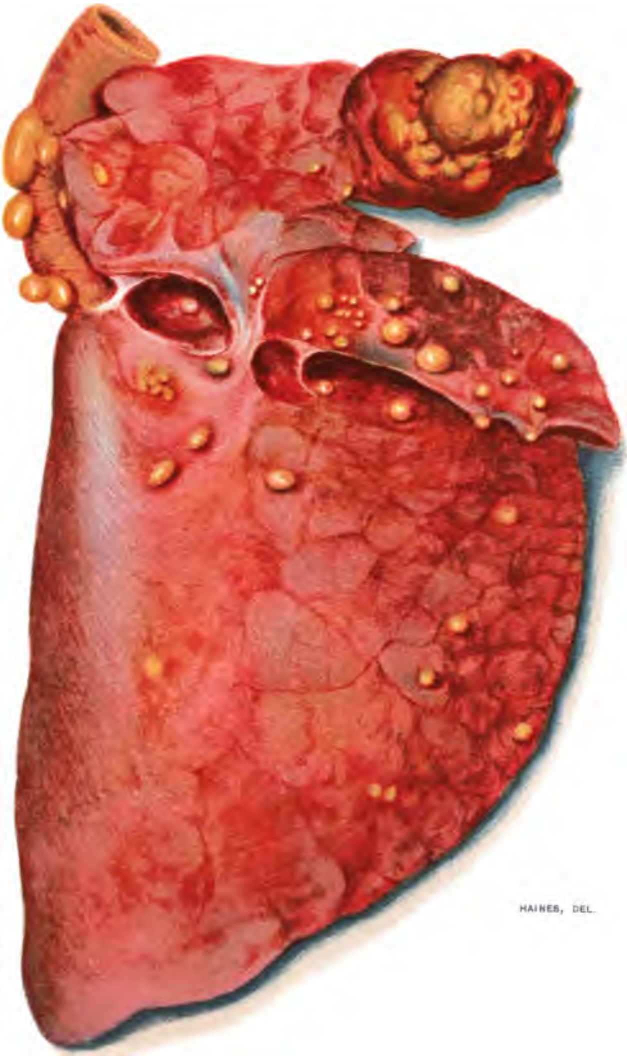
Ravenel had already proved his BB culture infective for cattle, and we can safely add our culture Boy V to the list of cultures derived from human sources that have previously been shown by other investigators to possess pathogenic powers for cattle.



HAINES, DEL.

LUNG OF SHEEP NO. 104, INOCULATED SUBCUTANEOUSLY WITH HUMAN TUBERCULOSIS (BOY V).

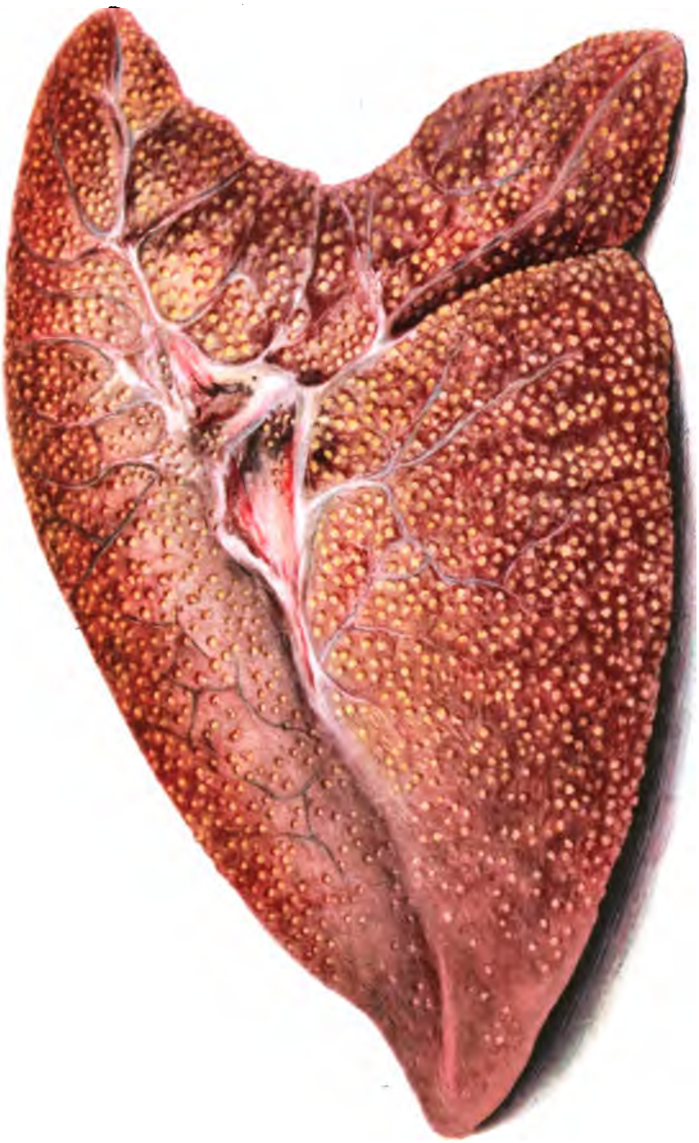
A Hoen & Co Baltimore



HAINES, DEL.

LUNG OF HEIFER NO. 302, INOCULATED SUBCUTANEOUSLY WITH HUMAN TUBERCULOSIS (BOY V).

A HOEN & CO BALTIMORE



HAINES, DEL.

MILIARY TUBERCULOSIS
AFFECTING LUNG OF HEIFER NO. 297, INOCULATED SUBCUTANEOUSLY WITH HUMAN
TUBERCULOSIS (B. B.).

A Hoen & Co. Baltimore

Of the 20 cattle submitted to the test, 3 failed to show any resulting tubercular lesion; 2 gave an extension of the micro-organisms no further than to the prescapular or to the prescapular and retropharyngeal glands; the remaining 15 developed tuberculosis of a generalized character. Out of these 15 animals, 3 died from the infection, and another was killed when in a moribund condition. It is of further interest to note that all of these fatalities were brought about by the use of infectious material derived from human sources, 2 direct from the culture and 2 from tissue emulsion after passage through cats.

The cattle inoculated were from 10 to 18 months of age, except a portion of those used in testing BB culture, for which purpose two animals were used that had reached an age of 20 and 22 months, respectively, and one—No. 318—that was 4 months of age. No young calves were used in any case. The tuberculin test was applied to each of the cattle, without reaction, previous to the experiment.

All inoculations were made subcutaneously on each side of the neck just in front of the shoulder, and 2 c. c. was uniformly introduced at each seat of injection except in the case of No. 340, Case XIV, where an accident necessitated the use of a smaller amount—1.25 c. c. on each side.

RESULTS OF CATTLE INOCULATIONS.

As has already been stated, culture Girl I failed to cause any form of tubercular lesion; neither was there any material rise in temperature resulting. The fourth generation of a thirty days' growth of dog-serum culture was used in the inoculation.

Culture Boy V, at the other extreme of virulence, caused tuberculosis of the severest type. The fourth generation of twenty-four days' growth upon dog serum was used for the initial inoculation, being applied to heifer No. 302. On the following day there was an elevation of temperature aggregating 2.8° F., and for about two weeks there was considerable fluctuation of temperature. After this the normal level of body temperature was gradually approached, but there was an evident disturbance of the animal's health. By the time five weeks had elapsed firm tumors had developed at each point of inoculation, the prescapular glands were plainly affected, and the general appearance of the animal indicated increasing unthriftiness. It was chloroformed on the one hundred and twenty-seventh day following the inoculation, and at the necropsy firm, irregular tubercular tumors 15 to 25 cm. in diameter were found at the points of injection. The adjoining lymph glands were swollen to the size of duck's eggs and supported several areas of hemorrhagic inflammation, also numerous foci of caseo-calcareous degeneration, while the retropharyngeal and submaxillary glands were slightly enlarged and inflamed, but contained no tubercles or bacilli.

A representation of the lesions of the lungs is given in Plate V. Many of the nodules on the visceral pleura projected from 1 to 8 mm. beyond the surface of the organ, and their similarity to the nodules frequently seen in "grape disease" of cattle, following natural infection, was very striking. The parenchyma supported numerous caseous areas, as indicated by the conglomerate, irregular nodular mass presented in the caudal lobe of the illustration. The numerous growths appearing upon the pleura were also suggestive of "grape disease." On the parietal pleura of the right side seven or eight tubercular nodules with constricted bases were located. The early stages of the disease were also apparent, as broad areas of hemorrhagic fibrous neoplastic growth were to be seen. On the left side similar fibrous neoformations were present, but the yellowish-gray projecting nodules were lacking. The diaphragmatic pleura likewise showed the early stages of this affection. The pericardium was also covered with small grapelike clusters and a hemorrhagic fringe of a tuberculous character. Numerous grapelike nodules appeared upon the serous covering of the thymus glands, especially upon the left side. Bronchial and mediastinal—especially the posterior—lymph glands were enlarged, edematous, and contained many calcified foci together with petechial hemorrhages. The liver supported some 20 tubercular nodules varying from 3 to 20 mm. in diameter. Five situated near the surface were especially worthy of note because of the fact that they projected some 6 mm. above the surface of the liver and were attached to it by constricted or pedunculate bases. Upon the peritoneal surface of the spleen a few irregular areas of fibrinous growth were present, with three tuberculous nodules attached to the splenic capsule. On section four additional foci were discovered in the parenchyma of the organ. An edematous condition was noticeable in the sublumbar and portal lymph glands, but no tubercle bacilli were demonstrated. No more complete invasion of the system by tubercle bacilli could have been expected had bovine bacilli been used.

The clinical history of the above animal applies equally well to the two following members of the series—Nos. 313 and 345. The former received 2 c. c. of an emulsion of the prescapular gland from No. 302 in front of each shoulder, while No. 345 was injected in the same manner with a similar quantity of an emulsion obtained from the prescapular gland of No. 313. There were no abrupt alterations of temperature, but in each instance a gradual increase of some three degrees was noted, followed by a season of fluctuation and afterwards by a slow return of normal conditions. The result of the post-mortem examination of No. 302 may with equal propriety be taken to represent the character of the post-mortem tubercular lesions shown by Nos. 313 and 345. Extensive invasion of the tissues by the organisms injected was disclosed in each instance, and a tendency toward the formation of grapelike bodies upon the pleura, pericardium, and upon the outer surfaces of the lungs and liver was also uniformly manifested. Prescapular, bronchial, and mediastinal glands were involved in both cases, as was the parenchyma of the liver and lungs. No. 345 also showed numerous fibrinous growths on the capsule of the spleen.

That the virulence of Boy V culture has not been decreased by continuous growth upon artificial media for thirty-one generations over a period of three years is indicated by the result obtained with two young cattle by the subcutaneous injection of a 32-day-old culture of the above generation of this organism. On June 21 bull calf No. 458 was injected as previously described. On August 3 the reaction at the seat of injection had become so extensive that large, flat swellings were observed, and both prescapular glands had increased to the size of duck's eggs. The general condition of the animal was poor and its appearance dull and languid. On August 8 the calf was so weak that it could scarcely walk. It was found dead on the morning of August 15, fifty-five days after the injection. On autopsy, lemon-sized tumors were observed at the seat of inoculation, which on section were found to consist of a uniform mass of tuberculous tissue. The connective tissue surrounding the tumors was edematous and sprinkled with minute caseous tubercles from 1 to 3 mm. in diameter. The submaxillary, post-pharyngeal, and tracheal lymph glands were intensely congested and enlarged, and contained tuberculous lesions. The prepectoral, prescapular, bronchial, and mediastinal lymph glands also appeared greatly enlarged and entirely tuberculous. The lung was uniformly sprinkled with innumerable necrotic tuberculous masses from 1 to 5 mm. in diameter. The distance between these different masses was about 5 mm. The liver contained one tuberculous focus about 3 mm. in diameter on the convex surface. The portal lymph glands presented two or three minute tuberculous nodules. The intestines, mesenteric glands, spleen, and other organs and glands were normal, with the exception that the lymph glands generally were more or less edematous.

Heifer calf No. 462 was inoculated with material similar to that used on the preceding calf, and on the same date. By July 30 the swellings at the seat of injection had gradually increased until they were 5 by 8 by 10 cm. in size, and the prescapular glands were each about the size of apples. The appetite of the animal was capricious, its temperature fluctuating, and its general condition poor. The animal was found dead on August 29, sixty-nine days after the injection. On autopsy, two large tumors were observed at the seats of injection, about 8 by 10 by 10 cm. in size; both of these swellings, on section, were found to be composed almost entirely of tuberculous masses from one-half to 2 cm. in diameter. The prescapular glands were greatly enlarged and completely tuberculous. The lung was evenly sprinkled with innumerable tuberculous foci, from 2 to 10 mm. in diameter. The bronchial, mediastinal, prepectoral, and suprasternal lymph glands appeared very much swollen, and contained numerous caseous areas. The post-pharyngeal lymph glands were also enlarged and tuberculous. The spleen contained a few small foci of tuberculosis. The liver showed no disease, but the hepatic lymph glands were enlarged, edematous, and contained each from one to six small tuberculous areas. The precrural lymph glands appeared slightly enlarged and revealed a few small tuberculous masses. The superficial inguinal glands were greatly swollen, but contained no tuberculous lesions. This was in the strictest sense a case of completely generalized tuberculosis.

The bacilli used in the initial test of culture BB upon cattle consisted of a twenty-six days' growth on dog serum and had had a total growth under artificial conditions of about sixteen months. The fourteenth generation was the only one available, and although the culture evinced considerable pathogenicity for the bovine race when used in pure culture, it is possible that its continuous artificial cultivation had lessened this virulence somewhat. This conclusion seems justifiable when the results here obtained are compared with those gained by Ravenel a year previously. At each point of inoculation (in heifer No. 266) a firm tumor gradually developed, which ruptured spontaneously about three months after the inoculations were made. These continued to discharge more or less freely up to the time of slaughtering the animal. The material cast off had the appearance of thick, creamy pus, in which many fragments of caseous débris were mixed. Post-mortem examination one hundred and seventy-eight days after the injection disclosed open ulcers at each point of inoculation. The prescapular glands were badly tuberculous. On sectioning the swollen glands they were found to be filled with thick, creamy pus and broken-down glandular tissue. The retropharyngeal lymph glands showed several tubercular foci, and in the surrounding tissues many yellowish tubercular areas were noted. There was no extension to either the thoracic or the abdominal cavity.

A second heifer, No. 258, was at once inoculated with an emulsion of one of the prescapular glands of No. 266. The resulting lesions were very similar to those found in No. 266 in their extent, but instead of the moist, creamy pus which filled each tubercular area, dense calcareous deposits were present. There was, moreover, an extension into the peritoneal cavity, where a scattering of small tubercular growths was found attached to the capsule of the spleen.

In order to test the effect of rapid passage through cats upon the BB tubercle bacilli, the third heifer—No. 297—was inoculated with an emulsion of omentum from cat No. 43, the fifth cat in the series. The resulting disease was marked by its rapid onslaught and wide extension. There was an elevation of temperature of 2° within a few days, and then a period of comparative regularity at points varying between 102.8° and 104° F. After this, up to the time of the death of the animal, the temperature was regularly high, flesh and strength were lost rapidly, and a persistent cough was present. At the post-mortem examination firm tumors with caseo-calcareous contents were noted at the seats of inoculation. The prescapular glands were of the size of a man's fist and contained many areas of tuberculous degeneration. On viewing the thoracic cavity, the visceral and parietal pleuræ were found to be firmly adherent. In like manner, where the various lobes of the lung came in contact with each other, the adjacent surfaces of the visceral pleura had become almost completely fused by a fibrous connective tissue growth, which could only be separated by the application of force. Scattered here and there over the external surface of the pericardium were small masses of nodules, each mass containing from 5 to 12, while a chain of similar nodules extended over the entire length of the organ from base to apex along the right side. The bronchial and mediastinal glands appeared badly affected throughout. The lungs were nearly filled with tubercular nodules 2 or 3 mm. across. These nodules were more numerous in the anterior portion of the lung, where the tubercular tissue was more plentiful than the normal lung substance. (See Plate VI.) The organisms had also invaded the abdominal cavity, and the parenchyma of both liver and kidneys were thickly sown with nodules varying from 1 to 5 mm. in size. A few similar nodules were present in the spleen, and the mesenteric glands also showed wholesale invasion.

Carried to the second animal—No. 318—of this series the resulting disease was less acute, but was still of a very active type and caused pronounced elevation of temperature with rapid emaciation of the animal. The post-mortem examination held ninety-seven days after injection showed walnut-sized areas of tuberculosis at the seats of inoculation, with marked involvement of the adjacent prescapular lymph glands and dissemination to the thoracic cavity, where the mediastinal glands and lung tissue presented numerous small caseous foci of disease. The third animal—No. 342—injected with an emulsion of the prescapular gland from the above calf produced lesions very similar to those found in the first of the series, but they were of slower development, and were not accompanied by emaciation or by marked increase of body temperature. When chloroformed one hundred and twenty-five days later, soft caseous tumors the size of pigeon's eggs were present at the points of inoculation. The prescapular glands were irregularly enlarged and calcareous. The costal pleura was very largely covered with inflamed fibrous patches composed of coalescing tubercular growths. The diaphragmatic pleura and the pericardium showed similar growths. The lungs contained numerous superficial fibrous growths, and the lung tissue throughout was permeated by countless small inflammatory foci, varying in size from a pin point up to a millet seed. The bronchial and mediastinal glands were enlarged and studded with tubercular foci the size of millet seeds and mostly calcified. On the external surface of the rumen was an area 13 by 20 cm., over which round, smooth nodules, in size up to a pea, were scattered. The omentum and the peritoneum showed wide areas roughened by coalescing tubercular growths and fibrous neoformations. The liver supported numerous small glassy foci. The spleen showed inflammatory tubercular growths in size up to beans on both surfaces of the organ.

Culture Sputum C failed to cause any tubercular lesion when a pure culture twenty-seven days old and of the fourth generation was injected. When accentuated by passing through a series of rabbits, generalized tuberculosis followed its introduction beneath the skin of cattle. The first animal—No. 312—thus treated evinced an acceleration of respiration within fifteen days after the inoculation, and at the same time a temperature of 106° F. The animal became unthrifty, but did not at any time show great emaciation. It was chloroformed on the one hundred and fifty-ninth day. At the autopsy the points of inoculation were found to support tumors, some 20 mm. across, each with a center filled with thick, yellowish pus. The retropharyngeal, submaxillary, and prepectoral glands were all enlarged and edematous and contained numerous small centers of tuberculosis. The prescapular glands were seriously involved, containing many large foci of yellowish calcareous matter. The bronchial and mediastinal glands were gritty on section and were thickly studded with tubercular foci. The parietal pleura supported upon its costal and diaphragmatic surfaces many inflammatory fibrous tubercular growths. The lungs contained 12 or more scattered grayish tubercular foci, located both deeply and superficially. The omentum presented an area some 50 mm. broad, extending across its entire width, to which a fringe of fibrous tubercular growths was attached. The liver contained both superficial and deep tubercular lesions, and its portal lymph glands were enlarged, containing foci of tuberculosis.

Carried to the second of the series by the injection of an emulsion of the prescapular gland, similar conditions were caused in regard to temperature elevation, general course of the disease, and extent of tubercular lesions developed.

This animal—No. 341—was killed on the one hundred and twenty-second day, after having shown a previous high fever and a general loss of condition. At the necropsy each point of inoculation showed a tumor about 50 by 100 by 40 mm. in size. Both tumors were composed of masses of tubercular nodules some 2 mm. in diameter, hard and calcareous. The prescapular glands were about 65 by 50 by 100 mm. in size, firm and gritty. Their surrounding membranes supported numerous scattered nodules in size from millet seeds to small beans. In places the adjoining muscle tissue had been invaded by tubercle bacilli and numerous scattered foci among the muscle fibers had resulted. The lungs showed superficial growths upon all lobes, in size up to beans. By sectioning it was observed that the nodules were scattered all through the lung substance and most of them were calcified and encapsulated. The mediastinal glands appeared swollen, and contained numerous calcified tubercular nodules. The costal pleura showed areas which supported pearl-like growths, some of them being pedunculated; in places they were collected together in groups comprising 10 to 12 grapelike bodies. The pleural nodules varied in size from millet seeds to marrow beans, and numerous fibrous neoformations were also present. The spleen showed slight infection. The kidneys each contained a few scattered foci. The liver had several small foci, as did the portal lymph glands.

The inoculation of yearling No. 304 with bacilli of culture Case XIV, taken from dog serum, caused no marked elevation of temperature or perceptible impairment of health. At the post-mortem examination, held one hundred and twenty-five days after the inoculation, each point of inoculation was found to support an abscess some 60 mm. across, which was filled with creamy pus loaded with short, straight tubercle bacilli. The prescapular glands were double their normal size, edematous, and had been invaded by tubercle bacilli from the adjoining local lesion. The inoculation of No. 314 with material from the local lesion of No. 304 gave only negative results.

An emulsion from the lung of rabbit No. 884, injected with Case XIV bacilli, was next inoculated beneath the skin of yearling No. 340. Instead of the customary dose of 4 c. c. given to the other cattle, this one received but 2.5 c. c., owing to the overturning of the tube in which the emulsion had been placed.

The temperature rose from 101.6° to 106° F. within fifteen days, then gradually subsided almost to normal, where it remained without material change until the animal was killed. The general appearance of the animal was affected; it was not as bright as usual, and its appetite was only fairly good at the time of acme of the fever. Chloroformed on the one hundred and fourth day, fluctuating tumors some 50 mm. across were found at the seats of the injections, from which creamy matter bearing many tubercle bacilli was liberated on section. The prescapular glands were greatly distended through the formation of calcareous areas of a great variety of sizes. The mediastinal glands were swollen and were sown with scattered calcareous nodules the size of millet seeds.

An emulsion of Case XIV bacilli obtained from the lung of cat No. 60 was next used, and this material caused the death of yearling No. 343 in thirty-five days. The disease assumed the most acute form from the very outset. The temperature rose to 107° F. on the sixteenth day, and at no time afterwards receded below 106° F. until just previous to death. Throughout the course of the disease the appetite was impaired, the respiration affected, and the animal became dull and weak. At the post-mortem examination the picture of acute inflammatory tuberculosis was displayed. The two points of inoculation consisted of firm tumors, 40 by 75 by 100 mm., composed of newly formed fibrous tissue, permeated with small caseous foci. The lymphatic glands of the neck and throat were invaded by the infection. The axillary and prescapular glands were similarly infected. The costal pleuræ were roughened by many fibrous tubercular neoformations. The pericardium supported numerous tubercular growths in size up to millet seeds. The lungs were distended and filled by myriads of small tubercular foci, each surrounded by zones of inflammation. The bronchial and mediastinal glands were greatly enlarged, the latter about four times their normal size, and were filled with caseous foci and inflammatory areas. The liver, the spleen, and the kidneys were studded with miliary foci, and the omentum had sprinkled over its visceral surface many fibrous neoformations interspersed with small, round tubercular growths, in size ranging up to millet seeds. The internal inguinal glands were inflamed and showed several tubercular areas.

Some months after the inoculation of No. 343 a further test was made by inoculating No. 344 with a culture from dog No. 93. This was done as a means of comparing the virulence of tubercle bacilli of Case XIV after passage through a series of cats, exemplified by yearling No. 343, with the same culture after passage through a series of dogs, and later recovered in pure growth upon dog serum. Considerable difficulty was experienced in getting a growth started from the tubercular lesions of dog No. 93, and the material finally used upon heifer No. 344 was taken from a feeble growth upon dog serum.

At the post-mortem examination of No. 344, held one hundred and sixty days after the inoculation, all mediastinal glands were found to be affected throughout with caseo-calcareous tubercular centers, from which tubercle bacilli were readily recovered in pure culture. The lungs also supported small, yellowish, gritty growths scattered throughout both the deep and the superficial tissues of the various lobes. From these lesions pure cultures were also readily recovered, direct transfer to glycerin-agar even resulting in quite a vigorous multiplication.

In testing culture Bovine S, inoculations of the usual amounts were made in the manner adopted in all previous cattle experiments, using bacilli of the third generation and of thirty-six days' growth upon dog serum. A transitory elevation of temperature followed in a few days. Firm tumors developed at each point of inoculation, but the prescapular glands at no time appeared to be enlarged. It was found at the post-mortem examination one hundred and sixty days later—the animal having been chloroformed—that the seat of injection on the right side had developed a small elongated subcutaneous abscess, which was filled with soft, creamy pus. On the left side was a cystic tumor some 50 by 50 by 100 mm., filled with watery pus. The right prescapular gland contained a single circumscribed tubercular abscess some 16 mm. across, filled with yellowish, cheesy pus. In the opposite gland three abscesses with similar contents had formed. These lesions were about 12 mm. in diameter. The mediastinal glands were slightly increased in volume and contained a few scattered caseous foci about the size of millet seeds. No other lesions of tuberculosis could be found in the carcass.

SUMMARY OF CATTLE INOCULATIONS.

The results obtained by the subcutaneous injection of these various tubercle bacilli into cattle indicate very conclusively that man may harbor tubercle bacilli of such pathogenicity as to cause the death of the cattle into which they are inoculated. The question of the apparent exalted virulence of several of these organisms after passage through a series of animals will not be discussed at present, but will be treated in another publication.

The negative effects following the injection of the culture of Girl I bacilli are in perfect accord with the results of all the other previously described experiments with these organisms. Culture Boy V again shows its apparent intimate relationship to the various bovine organisms, and in fact is much more pathogenic than the Bovine S culture. That the Boy V organism has retained this high degree of virulence over three years is another indication of the fixity of its character, and can not be accredited to the coincidence of extreme susceptibility of the two animals upon which it was last tested.

Cultures Sputum C and Case XIV were undoubtedly of such a low degree of virulence originally as to prevent their infecting cattle, but by manipulation it was found possible to cause a spreading tuberculosis in the animals inoculated.

Similar injections of the BB bacillus confirmed the results obtained by Ravenel in his intravenous and intraperitoneal inoculations of cattle with this organism, which placed it among the group of bacilli of the bovine type derived from children. The effect of the inoculation of Bovine S culture showed it to be less virulent for cattle than is usual with bovine cultures. Indeed, it may be considered an aberrant bovine organism, which leads to the inference that there is a certain range of pathogenesis possessed by bovine bacilli which is similar to, even if more limited than, the variation of virulence that we know exists among bacilli obtained from man.

Table showing results of inoculations of cattle.

[G denotes generalized or advancing tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of animal.	Material used for inoculation.	Age of animal. Mos.	Days alive.	Result.	Remarks.
Girl I.......	299	Culture..............	12	153	N.	Chloroformed; no lesions of any kind.
Boy V......	302	Culture..............	12	127	G.	Chloroformed; widely generalized tuberculosis.
	313	Lesion from 302.......	18	169	G.	Do.
	345	Lesion from 313.......	13	108	G.	Do.
	458	Culture..............	10	55	G.	Died; widely generalized tuberculosis.
	462	Culture..............	10	69	G.	Do.
BB.........	266	Culture..............	12	178	L.	Chloroformed; light infection; points of inoculation and prescapular and retropharyngeal glands alone involved.
	258	Lesion from 266.......	20	198	G.	Chloroformed; points of inoculation, prescapular glands, and splenic capsule affected.
	297	Lesion from cat 43	22	58	G.	Moribund; widespread tubercular infection.
	318	Lesion from 297.......	4	97	G.	Chloroformed; points of inoculation, prescapular and mediastinal glands, and lungs involved.
	342	Lesion from 318.......	16	125	G.	Chloroformed; widespread tubercular infection.
Sputum C..	300	Culture..............	12	158	N.	Chloroformed; no tubercular lesions found.
	312	Lesion from rabbit 741.	16	159	G.	Chloroformed; widely generalized tuberculosis.
	341	Lesion from 312.......	15	122	G.	Do.
Case XIV...	304	Culture..............	12	125	L.	Chloroformed; necrotic areas at seat of injection with slight extension to prescapular glands.
	314	Lesion from 304.......	15	119	N.	No lesions of disease found.
	340	Lesion from rabbit 884.	12	104	G.	Chloroformed; points of inoculation and prescapular and mediastinal glands severely attacked.
	343	Lesion from cat 60....	12	35	G.	Died; generalized invasion of visceral organs.
	344	Lesion from dog 93....	14	160	G.	Chloroformed; involvement of lungs and mediastinal glands.
Bovine S...	316	Culture..............	12	160	G.	Chloroformed; points of inoculation and prescapular and mediastinal glands involved.

HISTOLOGIC EXAMINATION OF LESIONS PRODUCED.

In the microsopic examination of the tubercular lesions observed in the various experiment animals used in this investigation, specimens of the lungs, the liver, and the lymph glands, and also of an occasional spleen and kidney, were examined in order to ascertain if any differences could be detected in the alterations produced as a result of the various kinds of tubercle bacilli used in these experiments. Numerous sections were made from the tissues of the animals in the various series, and in most cases all of the above-mentioned organs were obtained when diseased for the purpose of a comparative histologic study. In the case of dogs the tissues examined were chiefly the lungs, as the disease failed to spread to other organs in many cases. In cattle, goats, and sheep the lesions of the lungs, livers, and lymph glands were the ones principally studied, while in cats and rabbits all of the first-mentioned tissues were examined. It will be almost impossible to give any satisfactory description of the lesions observed in mice, as the effect of injection on these animals was irregular and lacked uniformity.

In all cases the tissues were embedded in paraffin after having been hardened and fixed in Zenker's fluid or in increasing strengths of alcohol. Several methods of staining were adopted, including alum-hematoxylin and carbol-fuchsin, hematoxylin and eosin, and Weigert's fibrin stain; but where the tubercle bacilli were to be brought out for comparative study the sections were always stained in carbol-fuchsin, decolorized in 20 per cent sulphuric acid, and counterstained in alkaline methylene blue, as previously stated. This examination revealed a varied picture, depending not only upon the character of the organism injected but upon the age of the lesions and the susceptibility of the animal to the material inoculated.

A determination of such differences as existed was readily made with bacilli from certain sources, but in a number of cases the extent of the lesions, the number of tubercle bacilli present, the amount of caseation observed, or the formation of fibrous tissue was so similar as a rule that it was impossible to note any uniform variations. For instance, it might be stated that in the majority of sections made from the lungs and livers of the series of animals inoculated with the bacilli from culture Sheep M, the largest sized tubercles, the greatest caseation, and the most numerous tubercle bacilli were observed. On the other hand, in those tissues obtained from the Monkey and Girl I cultures the other extreme was quite noticeable. In the lymphatic glands of these various animals tubercle bacilli were more numerous than in the viscera, and it was more difficult to notice differences in these tissues. In attempting to classify the lesions in groups the difficulty is at once met of having all gradations of the disease present in some cases or excessive alterations apparent in a number of animals of a series, but probably only slight lesions in a more resistant animal in this same series. However, such exceptions only prove the rule and will not be taken into consideration in arranging the cultures in groups.

The lesions of animals inoculated with cultures Hog O and Boy V were only secondary to those caused by the sheep bacillus in extent, in the unmber of tubercle bacilli, and in the activity of the cells surrounding the areas. The next gradation in the quality and quantity of tubercles produced includes, in the order of their severity, BB, Bovine S, Sputum C, and Case XIV. In fact, it might be stated that in this group the BB and Bovine S germs produced lesions slightly in excess and more active, with a greater number of bacilli present, than did the Sputum C or Case XIV, although in many cases—especially in the lungs and livers of rabbits and cats—a great similarity was noted in the lesions produced by all these bacilli, while in the kidneys and spleens the first two of this third group presented more advanced lesions and contained a more generous supply of bacteria.

In describing the lesions observed in the animals receiving cultures Monkey and Girl I, the livers and lungs of cats and rabbits are principally referred to, inasmuch as these organisms failed to infect mice, cattle, dogs, or, to any extent, sheep. The sectioning of necrotic areas in the above tissues shows numerous foci varying in size and due to cell infiltration, including all three of the constituents described as being typical of the tubercle—epithelioid, lymphoid, and, occasionally, giant cells. The larger tubercles consist mainly of epithelioid cells, while lymphoid cells occur at the edges in fair numbers. At the margins of these large areas there is occasionally seen an attempt on the part of the capillaries to enter in order to organize the structure. Caseation may or may not be observed in the central portion of the tubercle, and if present it does not progress to any marked degree. Tubercle bacilli in scant numbers are observed in these tubercles, either free, lying in the caseous matter, or at times within the giant cells or an epithelioid cell. Occasionally several polymorphonuclear leucocytes are seen in varying stages of granular degeneration in the caseated center. In some cases this area of caseation has failed to reveal a single organism after prolonged search, while in other instances bacilli are seen in the process of disintegration, as indicated by the light pinkish tinge of staining.

Directly in contrast to the slight destruction of tissue, the scarcity of tubercle bacilli, and the lack of activity of the cells surrounding the above tubercles are the more acute lesions induced by the bacilli of culture Sheep M. In the organs of animals inoculated with this bacillus the tubercles were large, with well-marked caseated centers, a tendency to progress, and with myriads of tubercle bacilli in the noncaseous as well as in the caseous matter. In fact, in many cases tubercle bacilli and epithelioid cells could be seen in the lymph spaces and occasionally in blood vessels in the apparently normal surrounding tissue.

Giant cells may or may not be found, but there appears to be a greater number observed in lesions produced by the bacilli of lower virulence than by this culture. These tubercles are probably the most extensive, most acute, and richest in tubercle bacilli of any. The tissues used in this comparison were the lungs, livers, spleens, and kidneys of cats, rabbits, sheep, goats, and mice.

The histologic examination of the lesions produced by cultures Hog O and Boy V were so similar in character that they will be discussed together. These acute, progressive tubercles partake largely of the nature of lymphoid cell infiltration with rapid coagulation necrosis of the central portion. Numerous tubercle bacilli are observed in the caseated centers, from which they may become transplanted on adjoining tissue through the agency of phagocytes, or by the lymphatic vessels. In fact, in these lesions many tubercle bacilli were observed in the lymph spaces of the apparently normal tissue surrounding the tubercle, where they may cause the development of other tubercles. That this has already occurred in certain cases was manifested by the appearance of an occasional single large tuberculous area composed of several similar tubercles. These tubercles also increase in size by peripheral growth as well as by this formation of daughter tubercles in the adjacent tissues. In the extent of the tubercles produced and in the amount of caseation present it was very difficult to differentiate these lesions from those produced by the sheep culture, but regarding the quantity of bacilli present it might be stated that while the amount was excessive in the lesions of both these cases there did not appear to be the myriads of bacilli that were noted in the sheep-culture lesions.

In the alterations produced by cultures BB, Bovine S, Sputum C, and Case XIV there were many tissues in which they all seemed to have about the same extent of alterations, while in others, especially noted in the kidneys and lungs of certain cats and rabbits, it was observed that the BB and Bovine S cultures produced a somewhat more extensive tubercle with greater caseation and a larger number of tubercle bacilli present than did Sputum C and Case XIV, but not to such an extent as to be included in the group occupied by cultures Hog O and Boy V.

It was thought inadvisable to compare the microscopic appearance of the lesions produced in dogs by Case XIV culture with those caused by the other organisms. For some inexplicable reason culture Case XIV had a peculiarly active virulence for dogs, producing lesions markedly in contrast to the other cultures of this group, or, in fact, even to those cultures (Sheep, Boy, and Hog) more pathogenic for other animals. In all tubercles in dogs, other than those produced by Case XIV, the tendency toward repair seems to have been stronger than the destructive agency, as indicated by the gradual transformation of the epithelioid cells into connective tissue elements. The small, glassy, translucent tubercles were completely circumscribed by the formation of connective tissue, which was also seen to have replaced in certain cases the central caseated area, causing the tubercle to be transformed by the connective and fibrous tissue cell infiltration.

It must be understood that in placing these various cultures in the above groups they were classified after taking into consideration the most advanced tubercles that could be detected in the various tissues. Of course it was frequently possible, even in the lesions caused by the sheep culture, to observe metastatic tubercles of cell infiltration of such recent origin that the amount of caseation, the number of bacilli, and the size of the tubercle would be far inferior to the well-developed and more aged tubercle produced by a less virulent culture, such as the Girl I bacillus.

CHEMICAL REACTION OF GLYCERIN-BOUILLON CULTURES.

In 1905 Theobald Smith[32] published the description of a simple method for distinguishing the bovine from the human type of tubercle bacilli in glycerin-bouillon cultures. Following closely the instructions there given, a number of the cultures already discussed in this article were made accustomed to growth upon fluid media and then tested as to their chemical reaction after growth in flasks upon glycerin-bouillon under the prescribed conditions.

Although marked differences are apparent in the adaptability of various cultures to growth upon these media, the chief interest is centered upon the effect of their continued cultivation upon the reaction of glycerin-bouillon. And from these effects, as stated by the author of the article just cited, " mammalian tubercle bacilli may be divided into two groups, one corresponding to the human, the other to the bovine type."

Cultures which adhere closely to the human type in their virulence for test animals and in their morphology will as a rule accommodate themselves to their new environment when transferred to fluid media with greater readiness than cultures of the bovine type, and the more luxuriant growth which is evinced by them when grown upon solidified serum is also displayed in the thick, wrinkled covering which they form upon the surface of the fluid. Cultures whose members belong strictly to the bovine type are slow to show growth, and when at last the surface of the fluid is completely covered the layer formed by their growth is delicate and thin for some weeks and does not approach in profuseness the culture of the human type, except in cultures that have been for a long time subjected to artificial cultivation. The acidity of the glycerin-bouillon used in these tests varied before inoculation from 1.85 to 2.1 per cent acid to phenolphthalein.

The most satisfactory results from this method of testing will be obtained from cultures that have been cultivated for several generations upon artificial media and that have become thereby so modified that they grow readily when transplanted from tube to tube.

In the article above referred to we read:

> When flasks of glycerin-bouillon in layers 1½ to 2½ cubic millimeters deep are inoculated with scales of tubercle bacilli from glycerin-agar cultures the floating masses soon begin to expand, and after one or two months a complete membrane will have formed over the relatively clear bouillon. The bacillar masses which fall to the bottom at the outset increase but slightly in size. This mode of surface growth of tubercle bacilli was shared by all mammalian races I have examined after they had acquired a certain saprophytic habit, and it is well known to all those who cultivate tubercle bacilli for the preparation of tuberculin. If ordinary bouillon prepared from fresh beef with 3 to 5 per cent glycerin added be used, and if the acidity be made equivalent to about 2 per cent of normal acid, phenolphthalein being the indicator, the reaction of the bouillon during the formation of the membrane will take one of two directions. It may gradually approach the neutral point and reach an acidity or an alkalinity of about 0.1 to 0.2 per cent when the membrane is complete and remains so, or else the acidity may diminish during the first month, to increase again during the second and fluctuate more or less if the observation be continued, but it does not reach the neutral point. * * *
>
> The reaction curve of the bovine cultures moves steadily from the acid to the feebly alkaline level, where it remains. The latter is reached in some lots of bouillon as early as three weeks, in others somewhat later. Very rarely it does not reach it. The reaction curve of the human cultures is more complicated. During the formation of the membrane the acidity is gradually reduced and may fall as low as 0.3 per cent. After the membrane is completed and as it grows thicker and enlarges in all directions by pushing the margin up the sides of the flask or even down into the fluid, the acidity gradually rises again, and it may reach 2 per cent. More commonly it fluctuates between 0.5 and 1.5 per cent. In no instance have I seen any of these cultures become neutral or faintly alkaline to phenolphthalein.

A great variety of cultures have been tested by this method and a large number of flask growths have been carried through a sufficiently long period of time to give the resulting reactions shown in the accompanying tabular form. While we do not look upon this method as capable of determining with any degree of accuracy the origin of any particular culture, it will be noticed that Boy V in both instances approached closely to the neutral point, and that the four flasks of Hog O culture similarly reduced the acidity of the glycerin-bouillon quite materially. It may therefore be concluded that both these organisms are of bovine type, although one was isolated from the mesenteric gland of a child and the other from the mesenteric gland of a hog. Several of the flasks from Girl I culture also showed considerable reduction in acidity, but others of the same culture failed to react in this manner, and these cultures, together with the Sputum C and Case XIV growths, support the conclusions reached by Smith.

Table showing chemical reactions of tubercle bacilli in glycerin-bouillon. (Cultures used in present experiments.)

Culture.	Variety.	Date of inoculation.	Time required to cover surface.	Reactions on stated days after growth covered surface (+alkaline; −acid).							
			Days.	*Days.*	*P. ct.*	*Days.*	*P. ct.*	*Days.*	*P. ct.*	*Days.*	*P. ct.*
Girl I	Rabbit 1250	May 5, 1905	56	10	−1.3	24	−1.1	43	−2.0	68	−3.5
Do	Rabbit 1220	Feb. 9, 1906	49	10	−0.9	24	−0.4	41	−0.4	63	−1.0
Do	do	Mar. 12, 1906	23	10	−1.1	24	−0.6	41	−1.1	63	−1.1
Do	Cow 361	Jan. 2, 1906	36	14	−0.9	24	−0.8	36	−0.6	......	......
Do	do	Apr. 12, 1906	20	15	−1.3	37	−1.1	51	−0.8	......	......
Do	do	do	19	14	−1.2	36	−0.7	50	−0.4	......	......
Boy V	Dog 88	Jan. 6, 1905	55	12	−0.5	29	−0.25	49	−0.3	67	−0.35
Do	do	Nov. 3, 1905	49	10	−1.6	30	−1.0	51	−0.6	61	−0.2
Sputum C	Cow 341	Mar. 12, 1906	44	10	−3.0	20	−3.3	52	−3.2	......	......
Case XIV	Cow 344	do	32	15	−3.2	32	−3.0	54	−2.7	......	......
Hog O	Cat 7	July 3, 1905	49	15	−1.4	26	−0.8	40	−0.3	61	−0.2
Do	do	Dec. 6, 1905	37	15	−0.9	26	−0.4	36	−0.1	61	−0.1
Do	do	Apr. 5, 1906	64	10	−1.2	21	−0.7	30	−0.2	61	−0.4
Do	Cat 22	Jan. 30, 1905	45	12	−1.8	21	−1.0	30	−0.3	......	......

In addition to the cultures in the above table, which are those already described in this paper, a number of glycerin-bouillon flasks were inoculated with tubercle bacilli derived from various sources in order to ascertain their chemical effect on this particular medium. The list includes Möller's grass bacillus and bacilli obtained from various wild animals, a dog, a fowl, and four children.

Table showing chemical reactions of tubercle bacilli in glycerin-bouillon. (Cultures derived from various sources.)

Culture.	Variety.	Date of inoculation.	Time required to cover surface.	Reactions on stated days after growth covered surface (+alkaline; −acid).							
			Days.	*Days.*	*P. ct.*	*Days.*	*P. ct.*	*Days.*	*P. ct.*	*Days.*	*P. ct.*
Avian	Old stock	May 12, 1905	26	10	−1.0	25	−0.8	55	−1.5	61	−1.7
Do	Rabbit 1101	Jan. 30, 1905	24	10	−1.3	23	−0.9	50	−0.9	65	−0.6
Do	do	May 12, 1905	27	10	−1.7	25	−1.6	55	−1.6	65	−1.8
Grass bacillus.	Möller's	do	10	10	−1.7	19	−1.6	40	−1.9	68	−1.1
Bear	Old stock	do	3	14	−1.5	28	−0.8	49	−0.5	70	−0.0
Do	do	Nov. 20, 1905	3	14	−2.0	28	−1.0	49	−0.6	70	−0.1
Baboon	Menelik	May 12, 1905	31	10	−2.0	21	−1.1	35	−1.3	......	......
Monkey	Bedford	Nov. 7, 1906	43	10	−1.1	19	−1.2	40	−1.2	......	......
Do	do	Apr. 10, 1906	35	14	−0.8	30	−1.2	42	−1.0	......	......
Bovine	111	Dec. 6, 1905	22	10	−2.0	15	−1.0	30	−0.8	40	−1.0
Do	do	Dec. 22, 1905	10	11	−1.4	20	−0.7	30	−0.4	40	−0.4
Do	do	Mar. 12, 1906	10	10	−1.6	19	−1.2	32	−0.8	......	......
Canine	Stock	May 5, 1905	57	12	−1.8	30	−1.2	40	−2.0	55	−3.1
Deer	New Mexican	July 7, 1905	24	10	−2.3	28	−1.0	43	−3.0	54	−3.2
Do	do	Dec. 19, 1905	18	21	−1.3	30	−0.9	45	−0.6	53	−0.5
Do	European	Feb. 8, 1906	33	18	−1.2	30	−1.1	50	−0.9	......	......
Do	do	Mar. 28, 1906	34	18	−1.2	30	−0.9	50	−0.9	......	......
Do	Sambur	Feb. 1, 1906	49	10	−1.2	20	−1.2	35	−2.7	......	......
Coati	Coati	do	38	10	−1.0	20	−0.8	33	−0.06	......	......
Do	do	Apr. 9, 1906	22	16	−1.9	24	−1.6	40	−1.0	......	......
Do	do	do	19	17	−1.6	39	−1.3	......	......	......	......
Nilgau	Nilgau	Mar. 12, 1906	21	10	−1.3	28	−0.8	43	−0.8	......	......
Do	do	Apr. 9, 1906	19	17	−1.4	30	−0.7	53	−0.6	......	......
Wallaby	Wallaby	Feb. 1, 1906	37	14	−1.2	22	−0.7	40	−0.4	......	......
Do	do	Mar. 29, 1906	42	14	−1.2	22	−0.7	40	−0.4	......	......
Do	do	do	38	10	−1.4	24	−0.7	31	−0.4	......	......
Human	Case 30, child	Dec. 14, 1905	58	10	−1.7	20	−1.2	30	−1.9	......	......
Do	do	do	24	10	−0.9	20	−1.7	30	−2.2	......	......
Do	do	do	44	11	−1.5	21	−1.5	34	−1.8	......	......
Do	do	do	44	13	−1.3	23	−0.9	33	−1.1	......	......

[illegible]

Culture	Variety	[illegible]	[illegible]	[illegible]
Human	Case 8, cerv[illegible]	[illegible]	[illegible]	[illegible]
Do.	Case [illegible]	[illegible]	[illegible]	[illegible]
Do.	do	[illegible]	[illegible]	[illegible]
Do.	do	[illegible]	[illegible]	[illegible]
Do.	do	[illegible]	[illegible]	[illegible]
Do.	do	[illegible]	[illegible]	[illegible]
Do.	Case [illegible], cer[illegible]	[illegible]	[illegible]	[illegible]
Do.	do	[illegible]	[illegible]	[illegible]
Do.	Case [illegible], cerv[illegible]	[illegible]	[illegible]	[illegible]
Do.	do	[illegible]	[illegible]	[illegible]

Referring to the above table [illegible] gives the reaction curves in cultures from miscellaneous sources, it becomes evident that uniformity of results should not be expected. None of the bovine cultures reached the neutral point. Bovine II is an old culture that has acquired during nine years' cultivation considerable saprophytism, but that this should not affect its reaction is shown by the reaction given by the bear culture which is still older and more saprophytic. It was confidently expected that some of the tests of deer, goat, nilgau, or walrus cultures would show the development of pronounced alkalinity, as the other tests had shown them to possess many characteristics of the bovine type, but this did not occur and human culture case 33 was the only one to prove distinctly alkaline and to react in a manner typical of the bovine bacillus.

It will thus be seen that this test while in a number of instances it shows a [illegible] relation between virulent tubercle bacilli and an alkaline reaction of the glycerin-bouillon, will not give a uniform reaction in a group of flasks which have been prepared and then sown in the same manner. Nevertheless there is an [illegible] to [illegible] it, and it is a striking fact that all the cultures derived from [illegible] human [illegible] in a way confirmatory of [illegible] results [illegible] exception of bovine [illegible] and case 33 cultures derived from [illegible] lymph nodes of children, whose difference in reaction [illegible] placeable on the ground of their bovine type. [illegible] Bovine III culture for some unaccountable reason [illegible] bovine reaction of these last two organisms [illegible] bacillus. The expectation that the [illegible]

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[1] Since these [illegible] tests have been made [illegible] cultivations [illegible] tests that cases 30 [illegible] Case [illegible] that case 33 [illegible] more [illegible]

bovine reactions was not realized, and may be explained by the fact that, coming from intermediary hosts, certain modifications of their biochemic characters have occurred, or that, having their own particular hosts, reactions peculiar to these hosts have been obtained, placing them in a group outside of either the human or the bovine class. Furthermore, these results would indicate that such chemical reactions are quantitative, not qualitative, and in cases where aberrant bacilli are studied irregular reaction curves may be expected.

CONCLUSIONS.

As a result of the foregoing experiments performed in this and other laboratories, we believe it justifiable to draw the following conclusions:

1. While certain peculiarities of growth, morphology, and pathogenesis are observed with a fair degree of constancy in bacilli of human origin, nevertheless these characteristics are not universal, and notable exceptions are observed which would confuse those who would attempt to establish their origin by means of such characteristics.
2. A similar degree of constancy in the morphological, biological, and pathogenic characters of the bovine bacillus is generally noted, but a certain range of differences has been observed, which, though apparently more limited than for the human bacillus, is nevertheless suggestive of aberrant forms.
3. Therefore the assertion, based solely on these facts, that a bacillus has a certain origin can only be tentative, as bacilli from man have been found which conform in all respects to bacilli obtained from cattle.
4. Tubercle bacilli of widely different virulence may be encountered in different cases of bovine as well as of human tuberculosis.
5. There is a certain proportion of cases of human tuberculosis in which may be found tubercle bacilli which are pathogenic for cattle.
6. Dependent upon the medium in which grown—whether in vitro or in vivo—tubercle bacilli will present special characteristics in accordance with their different environment, and these characteristics may lead to a supposition relative to their origin, although they are not always fixed or unchangeable.
7. The numerous instances above recorded in which the inoculation of cattle with tubercle bacilli from human sources has resulted in the production of tuberculosis must prove decisively that tubercle bacilli of human origin have been pathogenic to the bovine, or, on the other hand, the bacilli must have been bovine in origin, and in spite of this fact were readily able to obtain a footing within the

human tissues, in either case demonstrating the transmissibility of the differing types from one species of mammals to another.

8. The more the subject is studied the more numerous are the instances noted in which the bacilli appear naturally in animals that are far removed from the species supposed to be their usual host, thus proving that they are not closely limited in their choice of victims and that there is no character possessed by any of these types of tubercle bacilli in one host that may not be found in another host under favoring conditions.

9. Upon examination of the results obtained from inoculating bacilli marked " Boy V " in this experiment it will be at once noticed that it possesses a remarkable degree of virulence, in this respect surpassing many that we have obtained from bovine sources. Culturally, morphologically, and pathogenically this culture conforms closely to the bovine type. Could it be shown to have originated, previous to its attack upon the boy, from a bovine source, its fatal effects upon the child's system would offer incontestable proof of the susceptibility of the human organism to the inroads of the bovine tubercle bacillus. If, on the other hand, the bacilli for many generations past have successively grown upon human hosts only, we must at once admit that in this instance the type approaches so closely to the bovine form that all of the prominent characteristic differences between them have been removed, and we have before us a tubercle bacillus of human origin that has become so modified by peculiar environment that it is no longer a representative except in name.

10. There are human types of bacilli that are similar in morphology and biology, but vary as to their virulence; and, conversely, there are bovine bacilli typical as to form and growth, but less pathogenic than those usually observed.

11. If we exclude culture BB, which has already been included in Ravenel's description of bovine organisms obtained from human tissues, it will be observed that of four cultures obtained from children, one gave all the known reactions to the various tests applied for placing it in the bovine class.[a] It will also be noted that of nine cultures of bacilli obtained from sputa, one was entirely beyond the virulence possessed by the others, although not so pathogenic as some organisms coming from bovine animals, but nevertheless indicating the difference in degree of virulence among sputa bacilli. Of the eight germs obtained from cattle, hogs, and sheep, one was

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[a] Cultures of tubercle bacilli from the mesenteric lymph nodes of five children affected with infantile tuberculosis have been studied since the above work was performed, with the result that one was found of the bovine type, thus making two out of the nine cases of tuberculous children studied in this laboratory that were affected with bovine tubercle bacilli.

observed which had a modified virulence when compared with the high pathogenicity of the others tested, again illustrating the variability of virulence of bovine germs.

12. The question of the transmissibility of tubercle bacilli of bovine origin to man will not be here further discussed from these experiments; but sufficient evidence, in our judgment, has been adduced to warrant the adoption and enforcement of sanitary measures against the use of the meat and the milk of tuberculous animals, and to make it advisable to eliminate all tuberculous cattle from the herd or to sterilize all the milk therefrom. While the greatest amount of tuberculosis in man is undoubtedly caused by its spread from human to human, the frequency of his infection from animals should not be underestimated.

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PROCEEDINGS OF A CONFERENCE OF FEDERAL AND STATE REPRESENTATIVES

TO CONSIDER PLANS FOR

THE ERADICATION OF THE CATTLE TICK,

HELD AT

NASHVILLE, TENN., DECEMBER 5 AND 6, 1906.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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BUREAU OF ANIMAL INDUSTRY.—BULLETIN No. 97.

A. D. MELVIN, CHIEF OF BUREAU.

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Pathological Division: JOHN R. MOHLER, chief; HENRY J. WASHBURN, assistant chief.

Quarantine Division: RICHARD W. HICKMAN, chief.

Division of Zoology: BRAYTON H. RANSOM, chief.

Experiment Station: E. C. SCHROEDER, superintendent; W. E. COTTON, assistant.

Animal Husbandman: GEORGE M. ROMMEL.

Editor: JAMES M. PICKENS.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., January 15, 1907.

Sir: I have the honor to transmit herewith a report of the proceedings of a conference of Federal and State representatives which was held at Nashville, Tenn., December 5 and 6, 1906, to consider plans for the eradication of the cattle tick. The papers and discussions have an important bearing on the work now in progress by cooperation between this Department and the authorities of certain States for the extermination of the tick which spreads Texas fever of cattle, and it is desirable to disseminate this information among the people of the affected sections of the country. I therefore recommend the publication of the report as Bulletin No. 97 of this Bureau. For the sake of brevity the proceedings have been somewhat condensed.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.
Organization 7
Remarks by the chairman 7
Persons present 8
Order of business 9
The work of tick eradication from the standpoint of the Bureau of Animal Industry. By A. D. Melvin, Chief of the Bureau 10
Report of cooperative work on tick eradication. By R. P. Steddom, chief of Inspection Division, Bureau of Animal Industry 12
  The work by States 14
  Summary 19
Disinfection of cattle and premises. By H. A. Morgan, director Tennessee Agricultural Experiment Station 20
  Discussion 21
The legal side of the tick-eradication problem. By George P. McCabe, Solicitor, Department of Agriculture 28
  Discussion 32
Cooperation between the States and the Federal Government. By D. F. Luckey, State veterinarian of Missouri 35
  Discussion 36
The enforcement of quarantine regulations. By Thomas Morris, secretary Oklahoma live-stock sanitary commission 37
  Discussion 38
Education and publicity as an aid to tick eradication. By C. A. Cary, professor of veterinary science, Alabama Agricultural and Mechanical College 40
  Discussion 41
Interstate Live-stock Association 47
Reports from States 47
  Alabama 47
  Arkansas 47
  Florida 48
  Georgia 49
  Kentucky 50
  Louisiana 52
  North Carolina 53
  Oklahoma 54
  South Carolina 55
  Tennessee 56
  Texas 58
  Virginia 60
Remarks by Albert Dean on the work in the West 61
Work against Texas fever with special reference to Oklahoma. By Leslie J. Allen, inspector, Bureau of Animal Industry 66
  Discussion 70
Keeping records of inspections 71

	Page.
Feeding sulphur, dipping, etc	72
Difficulties of work along State boundaries	77
Trouble with ox teams	81
Remarks by W. H. Dunn	82
Plans, methods, and prospects for tick eradication in Texas. By Joseph W. Parker, inspector, Bureau of Animal Industry	83
Report on arsenical cattle dip for ticks. By Joseph W. Parker, inspector, Bureau of Animal Industry	89
Resolutions	92
Remarks in conclusion	93
Index	95

PROCEEDINGS OF A CONFERENCE OF FEDERAL AND STATE REPRESENTATIVES TO CONSIDER PLANS FOR THE ERADICATION OF THE CATTLE TICK.

ORGANIZATION.

The meeting convened in the Federal Building, Nashville, Tenn., December 5, 1906, at 10.15 o'clock a. m.

Dr. A. D. Melvin, chief of the Bureau of Animal Industry, United States Department of Agriculture, Washington, D. C., called the meeting to order and asked for nominations for chairman and secretary.

Mr. R. F. Wright, assistant commissioner of agriculture of Georgia, Atlanta, Ga., was elected chairman, and Dr. W. P. Ellenberger, inspector of the Bureau of Animal Industry, Nashville, Tenn., was elected secretary.

REMARKS BY THE CHAIRMAN.

Mr. Wright. I hope to have your hearty cooperation in the discharge of the duties of the important position to which you have elected me. We consider that no work in which the people of our grand country are engaged is more important than that of tick eradication, Six or eight years ago, in Georgia, we took this work under consideration, and with a little $500 appropriation we worked five or six long years and finally eradicated the tick from three counties. Since our National Bureau of Animal Industry, of which Doctor Melvin is chief, has taken charge of the work and given us some assistance, we have practically eradicated the ticks from seven counties of Georgia, and I think Doctor Payne, who has had the work in charge, will recommend three counties to be placed above the quarantine line provisionally. With a few exceptions, our people have taken hold of this work smoothly and systematically, and have allowed us to work straight through. They have given us their aid and cooperation. Doctor Payne soon organized his forces and got the work in good shape, and I am satisfied that in a year from now, say by the 1st of next August, six or seven of these counties will be ready to be placed above the national quarantine line; at least we hope so. But I would like to state that I think it would be a mistake to prematurely withdraw the forces from the States or from the counties in which they are now engaged. It may be that the appropriation has grown very small, and perhaps it will be wise to curtail the number of inspectors in our State. We have 10 or 12, I believe, but we would not be satisfied for the Bureau of Animal Industry to leave us until we learn to stand alone and to walk a little. If you remove these inspectors now, we can not expect to find things in good condition when we resume work next spring. On the other hand, if you give us an inspector for two counties and let him look them over during December, January, February, and March, we can still hold those counties in check.

We need a large appropriation. Our legislature had adjourned prior to last August when we began this work in Georgia. Owing to the great multiplicity of work before our State at that time, and the number of appropriations, and the confusion of a hot gubernatorial contest, the legislature did not make an appropriation; but I will assure the Department that next summer Georgia will come forward with a good appropriation to help this tick eradication work. A number of citizens of towns just below the tier of counties in which our work has been done have petitioned us to come down and see them. They are getting interested in the work. I am satisfied with the work which the Bureau of Animal Industry has done already. The greatest difficulties that are to be overcome in tick eradication in Georgia have been encountered in the manor districts where there is free pasturage and no stock laws. It was hard to manage them, and we have had a lot of trouble; but most of the counties still lower down have stock laws, and it will be much easier to eradicate the tick there.

I now declare this meeting ready for business.

PERSONS PRESENT.

The roll of States was called, and the following responded:

Alabama: C. A. Cary, professor of veterinary science, State Agricultural and Mechanical College, Auburn.

Arkansas: W. Lenton, veterinarian to State Agricultural Experiment Station, Fayetteville.

California: Charles Keane, State veterinarian, Sacramento.

Florida: C. F. Dawson, veterinarian to State board of health, Lake City.

Georgia: R. F. Wright, assistant commissioner of agriculture, Atlanta; C. L. Willoughby, dairy and animal husbandman, State Agricultural Experiment Station, Experiment.

Kentucky: F. T. Eisenman, State veterinarian, Louisville.

Louisiana: Wilmon Newell, secretary State crop pest commission, Baton Rouge; C. E. Mauldin, inspector, Bureau of Animal Industry, New Orleans.

Missouri: D. F. Luckey, State veterinarian, Columbia.

North Carolina: Cooper Curtice, inspector, Bureau of Animal Industry, Raleigh (representing State department of agriculture by request).

Oklahoma: Thomas Morris, secretary live-stock sanitary commission, Guthrie.

South Carolina: L. A. Klein, veterinarian to State Agricultural Experiment Station, Clemson College.

Tennessee: H. A. Morgan, director of State Agricultural Experiment Station, Knoxville; R. H. Kittrell, State live-stock commissioner, Murfreesboro; Zach. Biggs, deputy State live-stock commissioner, Trenton; John T. Goodrich, deputy State live-stock commissioner, Fayetteville.

Texas: J. W. Parker, inspector, Bureau of Animal Industry, San Antonio (representing State live-stock sanitary commission by request).

Virginia: John Thompson Brown, chairman State board of control, Brierfield; E. J. Harrison, Cumberland.

The Bureau of Animal Industry, United States Department of Agriculture, was represented by the following:

A. D. Melvin, chief of the Bureau.

R. P. Steddom, chief of the Inspection Division.

B. H. Ransom, chief of the Division of Zoology.

Cooper Curtice, inspector in charge of Virginia and North Carolina.

A. J. Payne, inspector in charge of South Carolina and Georgia.

W. P. Ellenberger, inspector in charge of Kentucky, Tennessee, Alabama, and Mississippi.

Albert Dean, live-stock agent in charge of Arkansas, Texas, and Oklahoma.

C. E. Mauldin, inspector, New Orleans, La.

Joseph W. Parker, inspector, San Antonio, Tex.

E. M. Nighbert, inspector, Georgia.

Willis B. Lincoln, inspector, Nashville, Tenn.

George P. McCabe, Department Solicitor.

ORDER OF BUSINESS.

Doctor **Keane**. I think the object of this meeting should be stated. It affects the whole southern portion of this country, and we should have a regular order of business. A committee on resolutions should be appointed by the chair and a programme of reports from the different States and the Bureau inspectors should be made out. I move that the chairman appoint a committee of five on resolutions.

The motion was seconded and passed, and the chairman appointed the following committee: Thomas Morris, Charles Keane, L. A. Klein, H. A. Morgan, J. Thompson Brown, and D. F. Luckey.

A motion by Mr. McCabe, seconded by Professor Willoughby, that a committee of three on order of business be appointed by the chair, and that the meeting take a recess of fifteen minutes in order to give that committee time to report, was adopted. The chairman appointed George P. McCabe, Wilmon Newell, and R. P. Steddom.

The committee on order of business submitted the following report, which was adopted:

1. Committee on resolutions to report, the report to be considered at any time.
2. Remarks by Dr. A. D. Melvin on "The work of tick eradication from the standpoint of the Bureau of Animal Industry." Fifteen minutes.
3. Report of Dr. R. P. Steddom on the work of tick eradication which has been accomplished to date. Fifteen minutes.
4. Prof. H. A. Morgan on "Disinfection of cattle and premises." Fifteen minutes. General discussion, thirty minutes, each speaker limited to three minutes.
5. Mr. George P. McCabe, "The legal side of the tick-eradication problem." Fifteen minutes. Discussion, thirty minutes; three minutes to each speaker.
6. Dr. D. F. Luckey, "Cooperation between the States and the Federal Government." Fifteen minutes. Discussion, thirty minutes; three minutes to a speaker.

7. Hon. Thomas Morris, "Enforcement of quarantine regulations." Fifteen minutes. Discussion, thirty minutes; three minutes to a speaker.
8. Dr. C. A. Cary, "Educational and publicational work."
9. Reports of State representatives, five minutes to a man.

(Signed) George P. McCabe,
Wilmon Newell,
R. P. Steddom,
Committee.

The Chairman. Unless there is objection, this report will be considered adopted, and it will be the order of business, unless otherwise changed by the meeting. Remarks by Doctor Melvin are now in order.

THE WORK OF TICK ERADICATION FROM THE STANDPOINT OF THE BUREAU OF ANIMAL INDUSTRY.

By A. D. Melvin, *Chief of the Bureau.*

Mr. Chairman and Gentlemen: I feel that this meeting is of the greatest importance, because it represents one of the greatest movements ever started for the betterment of the live-stock interests in the South. The deliberations of this body should carry a great deal of weight in outlining our future work. The work that we have done during the past years and are now doing has, I think, progressed sufficiently to show that tick eradication is practicable, and, considering the work that has been done, it is not expensive. It is very cheap, indeed, compared with the results and benefits that will be derived from it. Probably we ought not to be too sanguine as to our future progress, because the portions of territory that we have commenced upon will naturally be those that will be the easiest cleaned of infection. At the same time I do not think that we should be pessimistic; in fact, I am very optimistic over what we have done.

Before Congress took any action in making an appropriation for this specific purpose, I commenced to outline our future work. Doctor Steddom, as chief of the Inspection Division of the Bureau, was assigned to this work and nearly all of his other duties were assigned to others in order that this could have his undivided attention; and the carrying on and outlining of this work has been the greatest part of his work during the past year.

At the same time, through our Solicitor, we took up with the various States the question of legislation. This will be dwelt upon quite fully by Mr. McCabe. It was necessary for us to know what each State could do in the way of cooperation, as we could not do anything beyond regulating interstate shipments except through cooperation with the State boards. Some of the States had insufficient laws and some had no laws whatever to assist us; but we took advantage of everything there was and made the most of it, and we have gone ahead that way. But before we can make very great progress in some States, different legislation will be necessary.

Besides this legislation, each State should do all it can in the way of appropriation. I feel that the General Government must bear the greater part of these expenditures, but each State should do all within its power in the way of providing funds to assist, because the more money we have judiciously expended, the sooner we will get through with the work and the less the delay will be. The shorter the time in getting their farms and neighborhoods free, the less opposition we are going to have from the people. If for any reason it should be necessary to let the work drag along for several years, the people will become impatient and discouraged, and we will lose their support, which we must have, at least to a very great extent, to be successful.

The question of inspectors was one that embarrassed us a great deal, as in addition to this tick work we had the enormous meat-inspection work, which was provided for by Congress at the last session and which includes all slaughtering establishments or meat-curing establishments which do an interstate business, the only exceptions being retail dealers, butchers, and farmers. From having 150 establishments under inspection our service was increased to include more than 650 establishments, and instead of a force of something over 800 employees on meat inspection, we now have over 2,000. In order to extend this work it was necessary to scour the country for the necessary men. We were, and still are, lacking in number of veterinarians, and for this reason we could not supply for the work of tick eradication as many, and not always as experienced, inspectors as we desired. The States have assisted us considerably and we have been able in many cases to appoint State inspectors and pay their salaries. I think that the employment of many of those inspectors was a very wise thing, because they had the confidence of the people with whom they came in contact, and were efficient; but great care must be taken in the selection of all our inspectors. The mistake of one inspector or the carelessness or the indifference of another can reflect upon the work of all of them to some extent. People are inclined to charge the mistake of one individual to the whole force.

In addition to having efficient inspectors, we must try as far as possible to adopt certain rules and regulations upon which to proceed, and strictly enforce these regulations. The experience that I have had in all of our work has been that to have any favorites is a mistake. As far as possible, all individual cattle owners must be treated alike, circumstances and conditions being considered.

We have employed, besides the regular inspectors and persons located in the States whom we employed as inspectors, several others to visit and attend various live-stock meetings, in order to address them upon tick eradication, and not only instruct the people as to what we intend to do and could do, but, as far as possible, to create and encourage an enthusiasm for this work. We have had a number of these gentlemen, and I believe that their work has been very beneficial. It is necessary for all who are engaged in this work to familiarize themselves as thoroughly as possible with the subject and to talk tick all the while and never forget about it. Wherever you get a chance, where any possible good may come, talk about the subject and keep it alive.

The work that we have done, as indicated in the reports that we have received from our various inspectors, contemplates the releasing of a territory of something like 50,000 square miles. This is an immense area and includes territory in nearly all the States. It is an area, as the Secretary states in his report, larger than the State of Virginia. I think that we should feel very much gratified at having accomplished such a tremendous work in the comparatively short time of five months. Some of this territory may not be clean; we may still have to hold some of it in quarantine. But with such a showing as that I think we can go to Congress with assurance and ask for a larger appropriation in order that this work can be continued; and I believe that if we get the proper support we will make a greater showing next year than we have this year.

I came for the purpose of meeting you gentlemen, hearing from you regarding the work in your various localities, and seeing it from your point of view. Of course, we in Washington may have different ideas, and for this reason I desire to hear each one of you express himself.

REPORT OF COOPERATIVE WORK ON TICK ERADICATION.

By R. P. Steddom,

Chief of Inspection Division, Bureau of Animal Industry.

Mr. Chairman and Gentlemen: This is a report of the work up to October 31, 1906, submitted to the Secretary of Agriculture.

Under the provisions of the act of Congress approved June 30, 1906, appropriating $82,500 to enable the Secretary of Agriculture to undertake experimental work in cooperation with State authorities in eradicating the ticks transmitting southern cattle fever, the Department has for some months past been assisting the States and Territories from California to Virginia along these lines. Anticipating the action of Congress, an investigation was made respecting the laws of the various States, and through the various attorneys-general inquiry was made relative to the existence of State laws under which the Government could undertake the work of tick eradication. It was found that, while some State laws afford ample provisions, other States either have no law bearing on the subject or the statutes are inadequate. The following table shows the particular points covered by the investigation and the general trend of the information received:

Provisions of State and Territorial laws relating to live-stock quarantine and sanitation.

State.	Questions and answers.			
	Are local officers authorized and empowered to enter premises to inspect live stock and enforce quarantine, including counties, districts, farms, and ranches, and to control the movement of live stock?	Are such officers empowered to enforce such disinfection of animals and premises as may be necessary?	Are State officials authorized to issue rules and regulations establishing and maintaining quarantine lines?	May the State confer authority upon Federal representatives to act as officials of the State in such matters?
Alabama	No live-stock quarantine or sanitary law.			
Arkansas	Peace officers may enforce quarantine lines fixed by law and by the United States.	No	No	
California	Yes	Impliedly	Yes	As county officials only.
Florida	No live-stock quarantine or sanitary law.			
Georgia	Yes; impliedly so	Yes	Yes	Not prevented.
Indian Territory	No law to cover these matters.			
Kentucky	Yes	Yes	Yes	Yes.
Louisiana	Yes; impliedly so	Yes	Yes	No.
Mississippi	No live-stock quarintine or sanitary law.			
Missouri	No	No	No	No.
North Carolina	Yes	No	Yes	Yes.
Oklahoma	Yes	Yes	Yes	Yes.
South Carolina	No law to cover these matters.			
Tennessee	Yes	Yes	Yes	Not prevented.
Texas	Yes	Yes	Yes	No.
Virginia	Yes	Yes	Yes	Yes.

The matter was early taken up with the proper officials in the States and Territories interested, and arrangements were made for the Department to cooperate with them to the extent that their respective laws would permit. Under these arrangements the work has been done in close cooperation with the local authorities, who were permitted to designate the counties or localities to be covered and to recommend for appointment as agents of the Bureau men acquainted with the local conditions in the respective localities.

The territory in which it was desired to operate was divided into five sections, as follows:

1. California.
2. Texas, Oklahoma, Missouri, Arkansas, and Louisiana.
3. Kentucky, Tennessee, Alabama, and Mississippi.
4. Georgia and South Carolina.
5. Virginia and North Carolina.

The work was organized as soon as possible after the passage of the law, but it was late in July before it could be begun at all, and even later before it could be taken up in some sections.

As the conditions in the different sections were widely divergent, the plans of procedure and methods employed necessarily varied greatly. In some States meetings were held, at which the subject of tick eradication was discussed with farmers, stockmen, and other interested citizens. These meetings were intended to be largely educational, but they gave an opportunity to petition State authorities for relief and to express preference for local inspectors, thus developing an enthusiasm and interest that can only come from a close personal identification with an enterprise of this sort. In some places it was necessary to employ inspectors who could live in the saddle and wield a lasso like a cowboy. These men worked in groups of about a dozen, each group having a cook and a camping outfit. They covered their territory systematically, roping and examining cattle wherever found, and informing the owners of infested animals of the most practical method of getting rid of the ticks. It was found advisable to buy a carload of crude petroleum (in barrels) for use in the treatment of infested animals. This oil was distributed and used under the immediate supervision of inspectors of the Bureau in the Southeastern States, and was doubtless the means of doing what could have been done in no other way, as the crude oil is difficult to obtain in small quantities and at points far distant from its production.

The following table shows by States the number of herds inspected (except in North Carolina and Virginia), the number of cattle inspected, the number found free of ticks, and the number found to be infested, a grand total of 548,844 cattle having been inspected:

Statement of inspection work to October 31, 1906.

State.	Inspections.				Number of counties.
	Herds.	Cattle.			
		Free.	Infected.	Total.	
Alabama	780	4	5,550	5,554	2
Arkansas	1,527	6,671	2,332	9,003	2
California	1,015	67,517	58,889	126,406	11
Georgia	4,474	10,053	6,365	16,418	7
Kentucky [a]	4,077	13,653	7,332	20,985	4
Missouri	126	3,000	1,430	4,430	3
North Carolina	....	7,203	1,632	8,835	3
Oklahoma	10,589	97,860	16,972	114,832	5
Tennessee [b]	6,317	23,204	15,840	39,044	17
Texas	410	86,682	99,175	185,857	17
Virginia	....	12,217	5,263	17,480	11
Total	29,315	328,064	220,780	548,844	82

[a] In addition, in Kentucky 1,396 herds and 6,904 cattle were reinspected.
[b] In addition, in Tennessee 822 herds and 4,174 cattle were reinspected.

The work is still progressing in some States, but will have to be discontinued about December 1 on account of the lack of funds.

THE WORK BY STATES.

Following is a résumé of the work done and the results accomplished in each State to October 31. The outlook for next season's operations, as expressed in recent reports from the men in charge of the different sections, is also touched upon.

California.—The work, covering the inspection of 1,015 herds containing 126,406 head of cattle, has been done by eight veterinarians and one agent, and was confined largely to the counties below the quarantine line from which shipments have been permitted after inspection, viz, San Luis Obispo, Madera, Fresno, Kings, Tulare, Kern, and Merced. Some work was also done in Santa Barbara County. Herds known by local authorities to be infested were first located and inspected; then herds adjacent to these were carefully inspected, the aim being to locate and quarantine as quickly as possible all infested herds. These are now being disinfected, and it is expected that with continued work along the lines followed during the past few months the counties of Merced, Madera, Fresno, Kings, Tulare, and Kern may be released from quarantine before the close of the present fiscal year, and that the counties of Santa Barbara, Ventura, Los Angeles, and San Bernardino may be placed within the provisionally quarantined area. This will be the more easily and more certainly accomplished if, as is hoped and expected, the next legislature of California enacts a more satisfactory law and makes a specific appropriation of funds to be used in connection with the Department in tick eradication work.

Texas.—On account of the lack of funds the State authorities were unable to employ men who could give their time to the experimental work in Texas, but the regular State inspectors were directed to cooperate with the Bureau to the extent that their other duties would permit. The work was not begun until the end of August, but great interest was shown in some sections, one man—the manager of a large estate—offering $50,000 on condition that the work be undertaken in his county and with the understanding that his cattle might be given an unrestricted northern market. Eleven Bureau employees were engaged in the work in seventeen counties, and their reports show the inspection of 410 herds containing 185,857 head of cattle. The indications are that by the end of the present fiscal year the counties of Childress, Hardeman, Wilbarger, Cottle, Foard, King, Stonewall, Borden, Howard, Glasscock, and Pecos will be so free from general infection that they may be removed from the provisionally quarantined area.

Oklahoma.—The governor of Oklahoma and the officers of the live-stock sanitary commission gave hearty support and assistance to Bureau employees in that Territory. The laws are favorable, the stock interests are large, and the necessity for aggressive work looking to tick eradication is keenly felt. A farm-to-farm inspection was made, and wherever infested animals were found the owner was instructed as to the best and most practicable method of treating the trouble. The plan, which was different from that followed in any other section, was, briefly stated, as follows:

Both local and Federal inspectors were experienced in the saddle and able to rope and confine a suspicious animal wherever found, either on the open ranch or in pastures, and make a careful examination for ticks. These men traveled by twos from ranch to ranch, locating all infected cattle and premises. At the close of the day instead of returning to town or even going to a ranch house they went to their own camp, which had been moved forward a convenient distance during the day. Twelve men and a cook were usually with a single camp outfit, and in this way they covered the country in a systematic manner. They were followed a few days later by an inspector whose business it was to see that the work of disinfection was being properly carried on.

As the laws provide that where the owner refuses to accomplish disinfection the same may be done by the sheriff, and the cattle be sold to cover the costs, it was comparatively easy to enlist cooperation of owners of infested herds. Seventeen Bureau employees and a number of local inspectors were engaged in the work in Oklahoma, and accomplished the inspection of 10,589 herds containing 114,832 head of cattle; and while but one county, Greer, is to be at once placed within the provisionally quarantined area, yet an immense amount of good work was done in other counties, and it is expected that by the end of the present fiscal year the counties of Payne, Logan, Oklahoma, Cleveland, and Canadian may be ready to be released from quarantine.

Missouri and Arkansas.—Southwestern Missouri and northwestern Arkansas, lying contiguous to the infected area of the Indian Territory, have themselves become infected, and some work was done in five counties in that section. Three men report the inspection of 1,653 herds containing 13,433 head of cattle.

Louisiana.—On July 13, 1906, a Bureau inspector was directed to make a general survey of the conditions with a view to ascertaining what work in the line of tick eradication might be attempted in Louisiana. The meat-inspection work at New Orleans prevented him from making the investigation until late in September. He reports to the effect that whatever is done there will have to be done in connection with the crop pest commission, which at present is not in favor of enforcing the quarantine of infected live stock and premises. He recommends the liberal distribution of literature for educational purposes, and this is now being done, 2,000 copies of Farmers' Bulletin No. 261 having been sent to the crop pest commission for distribution at the State fair.

A Bureau inspector has been instructed to make a thorough investigation of the Gulf country of Louisiana, as it has been represented that sections of it are free from fever ticks. If the report of his investigation bears out the representations that have been made, the necessary steps will be taken to permit the shipment of cattle from such sections without restrictions.

Representatives of the Bureau have recently attended a meeting of the Association of Official Entomologists of the Cotton Belt, at Baton Rouge, at which the subject of tick eradication was discussed. Since their return from that meeting one of them states that, when the cattle owners desire, Louisiana will be an easy State to clean up, as the cane fields offer especial advantages.

Tennessee.—Plans for a division of the work in Tennessee were agreed upon by a representative of the Bureau and the State live-stock commissioner, the counties covered by the deputy State inspectors to be finally investigated by Bureau inspectors with a view to their exemption from quarantine. Thirteen Bureau employees were engaged in the inspection work, and an additional man was employed, who visited twenty-two counties, conferred with county boards, addressed twenty-four meetings on the subject of tick eradication, and through the county press brought the subject to the attention of the general public.

The results have been most encouraging, as according to recent reports and recommendations the counties and parts of counties mentioned below may now be removed from the quarantined area: The counties of Moore, Cannon, and Cumberland; about one-third of each of the counties of Haywood, Fayette, and Coffee; about two-thirds of each of the counties of Madison and Jackson; about one-half of each of the counties of Clay and Dekalb, and a small portion of each of the counties of Franklin, Putnam, and Fentress. These sections are not entirely free from ticks, but the infested animals are few and are controlled by the State and county authorities under agreement with the Bureau, so that their proper treatment may be carried to completion without danger of spreading the parasites. Up to the end of October, 6,317 herds, containing 39,044 head of cattle, had been inspected, 822 herds of which, containing 4,174 head of cattle, were reinspected.

Kentucky.—For some years past it has been necessary to include within the provisionally quarantined area a few counties in southern Kentucky lying contiguous to some of the infected counties of Tennessee. The number so included had been reduced to two counties, Clinton and Wayne; but slight infection was also found in two adjoining counties. The entire matter was taken up with the State board of health, and that body on August 1, 1906, issued a proclamation placing a quarantine upon these counties and conferring upon the employees of this Bureau the authority of sanitary inspectors of the State board of health. Under this arrangement the counties were thoroughly covered, with the result that it is now recommended that they be removed from the provisionally quarantined area. This work was done by seven Bureau employees and included the inspection of 4,077 herds containing 20,985 cattle, and the reinspection of 1,396 herds containing 6,904 cattle. Under agreement with State and county authorities, the remaining infested animals and premises will be kept under quarantine until disinfection is complete.

Alabama.—In Alabama the work was largely of an educational character, instructing owners of cattle regarding the work of tick eradication and methods of disinfection. The work was done by three Bureau employees in two of the northern counties, Limestone and Madison, and included the inspection of 780 herds containing 5,554 head of cattle. The infection in these counties is general, and while there is no State law under which effective work can be done, the people are so alive to the situation and gave such cordial support to the work that there is reason to believe that permanent results may be accomplished if the work can be resumed early in the spring of 1907.

Georgia.—The work in Georgia has been confined to seven counties in the northeastern part of the State, as follows: Stephens, Habersham, White, Dawson, Pickens, Gilmer, and Fannin. It was at first largely educational and met with the hearty approval and cordial support of farmers, stock owners, and others interested. In addition to the educational work the thirteen Bureau employees engaged inspected 4,474 herds containing 16,418 cattle, and, while the percentage of infection is very high in some of these counties, in three—Stephens, Habersham, and White—it is so low, and the work of disinfection is progressing so well, that they can probably be placed within the provisionally quarantined area before the close of the present fiscal year. It appears that a large majority of the better and representative citizens of the counties covered have contributed their support to the work, and that if work can be resumed early in the spring of 1907 a number of other counties may be either added to the provisionally quarantined area or entirely released from quarantine.

North Carolina.—The cooperative work in North Carolina has been with the State board of agriculture, through its veterinarian and seven inspectors, the Bureau force averaging four inspectors and three agents. Meetings for the discussion of the question of tick eradication have been held, infested herds have been located and quarantined, and other preparations made that will facilitate an intelligent resumption of the work in the early spring. The farm-to-farm inspection was made in three counties, and the inspection of quarantined farms was made in six other counties. The total number of cattle inspected in three counties was 8,835, 1,632 of which were infected. Five whole counties—Polk, Forsyth, Davidson, Cabarrus, and Mecklenburg—and parts of two other counties—Rowan and McDowell—are recommended for release from quarantine, and three others—Rutherford, Yadkin, and Cleveland—are recommended for provisional quarantine.

Ten counties in the northern part of the State and three in the southern part have been agreed upon as working ground for the next season.

Virginia.—The cooperation has been between the State board of control and its veterinarian and the county authorities on the one hand, and the Bureau force, averaging about nine inspectors and four agents, on the other. The working force of the State has been forty-one local inspectors, whose employment has ranged from two weeks to three months each. The work was confined largely to eleven counties, though some inspections were made and some educational work was done in five other counties preliminary to next season's operations. The manner of the inspection has been to make a farm-to-farm canvass of the live stock, usually with the local inspector, and, if premises were infected, to ascertain if they were quarantined, and if not, to report the fact to the local inspectors. Such places were thereafter revisited periodically to see if disinfection was being properly accomplished, and in most cases it was found that this was being done. The number of cattle inspected was 17,480, of which 5,263 were infested, many of them being reinspected one or more times.

As a direct result of the season's work in Virginia, ten counties—Franklin, Campbell, Appomattox, Buckingham, Prince Edward, Amelia, Nottoway, Powhatan, and Mathews—with an area of 4,445 square miles, are to be released from quarantine on December 1, and two other counties may be placed within the provisionally quarantined area. There are some infested herds in the ten counties referred to, but they are quarantined, and under agreement with the respective county boards of supervisors they are to remain in quarantine until released by written order of the State veterinarian approved by the chief of the Bureau of Animal Industry.

If the work outlined for next year is accomplished, as may be reasonably expected, but nine counties will remain in the quarantined area of Virginia.

SUMMARY.

In considering the work done and the results attained thus far, it should be borne in mind that the season was well advanced before the law was passed, and that, although some steps were taken in anticipation of its passage, the actual plans and organization for the work were late in formation. It should also be remembered that the amount appropriated was only intended to be used to inaugurate the work, and yet, as set forth above, employees of this Bureau have inspected 548,844 head of cattle, and have, in connection with local authorities, so attended to their disinfection and to the supervision thereof that forty-five whole counties, and parts of thirteen other counties, with an area of almost 50,000 square miles, will probably be released from quarantine before the end of the fiscal year. This is an area larger than that of the entire State of Virginia. Plans are laid and specific work is outlined for resumption in the early spring. The State officers, cattle owners, and others affected are intensely interested; the educational work will be carried on, and there is every reason to believe that with proper funds at the disposal of the Department next season large inroads may be made into the territory now quarantined, and hundreds of thousands of cattle be given an unrestricted market, thus giving direct results to an immense number of people. This will stimulate interest in those States in which active interest is now lacking, and will doubtless result in a more general movement against the cattle tick.

If the Congress at its next session will appropriate $250,000 for extending these operations, and will continue to sustain them adequately, and if the States interested will do their part in the way of enacting favorable laws and appropriating money to be used in this cooperative work, it is only a question of time when the southern cattle tick in this country will be a thing of the past.

DISINFECTION OF CATTLE AND PREMISES.

By H. A. Morgan,
Director Tennessee Agricultural Experiment Station.

Mr. Chairman and Gentlemen: I will talk on just one phase of this question of disinfection—that associated with the knowledge of the life history of the tick.

It appears to me that we have three distinct tick areas or zones in the infected district of this country, based on temperature conditions. I believe that the crop rotation system of Tennessee will furnish an easy method of tick eradication upon absolutely natural lines. I was not familiar with the conditions existing in this northern territory until I came to Tennessee, and I had foremost in my mind those conditions which exist in Louisiana. We have a condition in Tennessee where the cattle pretty generally are free of ticks during the winter time. Now, associate that with the crop rotation system that is common in this State, and which is very similar to that in northern States. It takes in the hay crop. A farmer has a field of meadow from which he takes off a hay crop, and he can be careful to keep that field free of cattle for that season. When the winter time comes his cattle will become clean for the next spring. The same condition exists on down through Alabama, Georgia, and Mississippi, and to a limited extent in Louisiana, and in Texas. But a difference exists in

Georgia, Alabama, and Mississippi, because they have different systems of cropping. In this northern zone where we have the cattle tick off of the cattle during the winter time we have a tremendous advantage in that we do not have to disinfect the cattle. In Tennessee we have conditions that will permit easy eradication. Where we follow the rotation of the farmer, we drop into his methods. But when we get to Alabama and Georgia, where the hay crop does not exist and the pasture does not exist, the problem is a different one. Farther down, where we can look upon the tick as infesting cattle throughout the year, the problem of disinfection is an entirely different one. I believe that while the progress may be exceedingly rapid in this northern area, we are going to have more difficulty farther south. It is a question there of introducing winter crops for pasturage. What shall we do? There is no more available crop in that southern tier of infected States than oats. And I believe that the use of the oat crop in itself means tick eradication. The majority of the cattle are not under cover in winter, so we can turn them into the cornfield and the cotton field until they drop the ticks, then turn them on the oat field that will maintain them up to June. I believe that in this plan we have the line of least resistance in that territory.

Hence, to sum up, following the line of least resistance to the farmer, we have two distinct conditions—one in Tennessee, where we have no ticks on the cattle during the winter months, and another farther south.

DISCUSSION.

Doctor CARY. I have had the opportunity of traveling through Alabama for fifteen years, and have familiarized myself rather thoroughly with this rotation business. We have a problem there that is difficult, yet it is not out of reach of the practical farmer. We not only have this oat crop for winter and spring, but we have also the rye crop, sometimes the wheat crop, and sometimes the vetch crop in winter, and these afford good pasturage for spring and late winter and really bridge over that season, which is a great help to a system of rotation for the eradication of ticks.

Professor MORGAN. I am very much interested in this point. I should like to know if it is true that cattle are really freed of ticks naturally in the winter, and how far south that extends. I think that sometimes it extends to middle Alabama. Last winter was a very open winter, and ticks were on cattle up above the middle of Alabama and stayed on them all winter; but usually they are off down through middle Alabama for periods of one to two months.

Doctor DAWSON. We have ticks with us in Florida the year round. We have plenty of ticks on cattle in the winter time, but they are very much less virulent, and young animals have been brought to

Florida with comparative safety. Just about this time (December) is the time to bring animals in. Florida has done nothing at all toward tick eradication. This is the first recognition our State has taken of the tick question. I have no doubt that, when other States show what is being done, Florida will also get to work and free our cattle from the bad name which they have had. We do not have any reason to send cattle north, but after a while when the Cuban market drops out we have got to get into the raising of better cattle, and then will come up the question of eradication in Florida.

Mr. KITTRELL. Mr. Chairman, I indorse what the doctor said in regard to Florida. I have demonstrated what he has stated. I shipped a carload of cattle to Tallahassee, Fla. They were grown cattle, milch cows, breeding cattle, and I shipped them in the winter; but early in the spring, even before May, they had died with "murrain," as they call it. We did not know what killed them. I received another order for a few cows and sent them, and they also died. The son of one of the farmers then came up, and I sold him sixteen calves, four or five months old, and they were shipped down in December and all lived. That does show that the ticks are less virulent in Florida during the winter than in the summer. By summer time the cattle had become somewhat immune, and that enabled them to resist the tick.

Doctor KLEIN. In regard to South Carolina, we must figure on the tick living all the year round. In applying the rotation system of pasturage to South Carolina, including the use of the cultivated field, we meet there with a difficulty that is well-nigh insurmountable, and that is that the cultivated fields of South Carolina are not fenced. You can readily see the difficulty of using the rotation system there. It will be necessary to talk to the farmers and have them put up fences in order to get rid of the tick. They regard the fence as a great inconvenience. It takes up room and reduces the area of the land that they can put under cultivation.

The CHAIRMAN. How about the timber?

Doctor KLEIN. They have not much timber. I may say that in South Carolina I have found that the most practical method of disinfecting cattle and farms is by the use of crude Beaumont oil. Begin in the fall and apply the oil; let the cattle use regular pasturage and go about as usual; but apply the oil every time the ticks appear.

The CHAIRMAN. How often?

Doctor KLEIN. The first application should be made about the 15th of September. It may have to be applied again once before Christmas, and then, perhaps, once or twice in the spring. There is one farm that I cleaned up by the use of Beaumont oil with three applications, and did not begin until the spring. There is another farm on which I did not begin until the spring and which I cleaned up with four applications. I wish to point out another thing in regard to the use of crude Beaumont oil—it is the cheapest substance that the cattlemen can buy to disinfect cattle. The price depends entirely upon the quantity bought. If bought in barrel lots it will cost about 6 cents a gallon, or $3 a barrel. The freight by rail from Port Arthur, Tex., amounts to about $4 by the barrel. That makes about $7 a barrel for this oil. That is cheaper than a man can get crude cottonseed oil, and this Beaumont oil has an advantage over all others in this, that it remains on the cattle longer; its effects are more lasting than those of any other substance that I have ever seen put upon cattle. The lasting effect will be much greater if the oil is used in the fall and spring than if it is used in the summer. In the hot weather of summer time the effect is not so lasting as in the winter.

Professor MORGAN. What effect has this treatment on the cattle of South Carolina?

Doctor KLEIN. Practically no bad effect. I have used it on cattle in South Carolina in August.

Doctor CARY. Does it increase the temperature?

Doctor KLEIN. Not perceptibly. Nearly all our pastures have a little timber, and after the oil was applied the cattle kept to the timber—hunted the shade. Only two showed any injurious effects whatever, and they merely showed a very slight irritation of the skin—nothing that lasted more than one or two days. The greatest objection that I ever heard against the oil is the odor. The negroes objected to it, probably on account of its being an odor they are not familiar with. They would not milk a cow properly after she had been treated with this oil, and I have had several cattle owners complain that it reduced the quantity of milk. But if a cow is properly milked she will show no great decrease. There may be a little decrease, but no more than would occur from putting a cow in a strange barn.

Doctor LUCKEY. I believe that the oiling of the cattle with Beaumont oil or any other kind of oil will be the principal method of getting rid of ticks. In districts farther south I think it will be found almost impossible to move cattle from pasture to pasture without their picking up infection as they travel about. But if they are oiled with any sort of oil, the ticks can not possibly get on them. I know we can get very good results by oiling cattle. Six years ago we had eight counties rather badly infested with fever ticks. In 1904 there was scarcely an animal with fever ticks. We have accomplished the result by quarantining all the cattle that were infested with ticks and requiring them to be oiled by hand. We usually used West Virginia black oil—it was not so irritating as Beaumont oil. We commenced placing infested cattle in quarantine in dry lots, where we could find the space to place them, until they were disinfected by thorough hand dressing, which was done by pouring oil out of a bottle and hand dressing. We had 200 head disinfected in this way; and if later on they were inspected and found free, they were released, but if not free they were oiled again and placed in another pasture. We think those pastures will all be free from ticks in the spring. It was impossible to quarantine the outlying range, but we forbade the return of any disinfected cattle to the range. We depend on the use of some sort of oil. Leave cattle right on the ticky pastures and thoroughly oil them with lard or cotton-seed oil four times during the summer, and the ticks on that pasture will, in all probability, be gone by fall. Where rotation of pastures is impossible, this method is very important.

Doctor PARKER. In Texas as far north as the Red River—the extreme north of infection—I do not believe there is any season of the year that cattle on infected pastures will be free from ticks. I know in the winter time they are passed by inspectors, as they stand ordinary inspection, but this does not mean finding every tick on them. I doubt if in Hardeman County there is any time during the winter that ticks could not be found, if the pastures were pretty well infested. In fact, I have come to the conclusion that most of the ticks in northern Texas live during a large part of the winter on the cattle and not on the ground. This year we have had a very severe winter so far. On the 22d of November the temperature had gone down to about 5° above zero for four days. That is sufficient to get rid of all the large ticks on the ground, and on a pasture where the cattle had been freed of ticks by dipping, say, two or three weeks before that, there is very little probability of there being mature ticks on the ground. Only the eggs or larvæ would remain. Yet I think ticks will go through the winter unless the cattle are thoroughly dipped. The method we are pursuing there is first to free the cattle of ticks, if it is a small herd, by the application of Beaumont oil. About this time, when the wheat grows up enough to be pastured, run the cattle on that through the winter; the winter will have disposed of the infection of the cattle.

Doctor CURTICE. I am here representing the State of Virginia, in which the State veterinarian says that he would not use oil; he thinks that anotner way is better. The people themselves apply the oil insufficiently and indifferently and thus protract the work through one or two years. The State veterinarian does not doubt that farms may be readily cleaned by the careful use of oil. The farmers need to be taught how to take care of cows. It seems to me that the majority of southern planters do not love the cow; they do not usually have dairy cows or beef animals of the right type.

I have read somewhere—I am not speaking for the Bureau now—that experimental farms are to be started in the South; and I think that in certain localities the Bureau could do no better than to buy wire fences, fence up inclosures, produce grass, and disinfect the cattle in that way. The fences would be much cheaper than the continual reinspection. Where the Virginia farmers hire pastures, they have applied oil with some success. Eradication will lie between the use of fences and oil.

Doctor NIGHBERT. In visiting farms infested with ticks the greatest trouble I find is to get the cattle well greased when we give instructions to grease them. In nearly every instance the owner of a cow will begin at the wrong end. He always begins his work where he sees ticks—on the hind legs and on the flanks—while he should begin at the front end. I have never seen a cow's tail greased yet; and who has not seen a number of ticks on the tail? This is a very important member of the cow's body. The ticks get on it when the cow lies on the ground. I have never seen a cow well greased when it was left to the farmer. It will be necessary for the inspector to supervise, if the disinfection is to be carried on by greasing.

Professor NEWELL. We in Louisiana are not inclined to favor the use of oil very much, or at least not as one of the principal means for eradicating ticks. Louisiana is the hotbed of the tick in the South. Those of our cattlemen who have used oils not only have injured their cattle but have failed to clear off the ticks. Not long ago I saw a bunch of cattle dipped, but all the ticks were not eradicated. If you put these cattle on pasture I do not think you can ever get rid of the ticks on the pasture. If you do, it will not be for a long period of time. In Louisiana we expect to depend on starving out the tick, ridding the cattle of ticks by rotation systems which work with mathematical certainty. When you come to grease an animal you will find that there are a great many things to consider. If it happens to be a Texas steer I know the animal will not be properly greased. There is not one animal out of fifty in Louisiana that you can grease thoroughly by any hand method. In Louisiana we have free range to contend with everywhere, and at the same time we have a much heavier tick infestation. This furnishes an incentive for a man to fence his own property, because, on account of the loss by the parasitism of the ticks, a man is perfectly willing to put up the fences when he learns that in this way he can get rid of the enemy.

Mr. MORRIS. I recommend the Oklahoma system, and when it comes to greasing, we grease the animals. We rope the steer and throw him, and grease one side, and then turn him over and grease the other. You can not grease a steer by letting him stand up or by putting him in a chute. When you throw him you can grease him well.

Professor WILLOUGHBY. How many a day can you grease that way?

Mr. MORRIS. Well, we grease a lot of them.

Doctor **Keane**. A remark made by the original speaker on this subject—Professor Morgan—about working along the line of least resistance appealed to me very much. You can not eradicate ticks by any general method. I have had considerable experience in California. You have got to treat infected ranches according to the conditions which exist there. You may find a little dairy herd where the ticks can be picked off. Then you may go to a large ranch where you have a different class of cattle which should be thoroughly dipped, as this is the only way you can reach all the ticks. In California we have a great many dipping plants. In one county we have about 40. We may select a point where we have considerable infection in a district and either establish a private dipping plant or get the county to put in a dipping plant. Then we can have these infected cattle dipped all in one day. We have what we call collective dipping. We inspect these cattle right along, at intervals of about three to five weeks, and before the ticks are large enough to drop off we dip again. We aim to prevent the large females from dropping off and laying their eggs. It is only a matter of time when the ticks will be eliminated absolutely. But you have got to be there and watch the dipping and see that it is done effectively and thoroughly. If not, there is always the possibility of some of the ticks escaping, thereby setting back the work. The rotation methods and taking cattle off of infected ranges for a year is the most practical way of eradicating ticks. We have cleaned up many ranches by that method, and we have cleaned them up by dipping. We force people to do the work in California. In regard to fences, we make the owners fence up the ticky cattle, and if they break out we prosecute them; and we have made it stick in every case that we have prosecuted.

We have a quarantine law and a law for entering premises. It does not give the power to disinfect animals, but we can prohibit these cattle from being taken out. Where a man finds he can not take his cattle out of his ranch, he will do anything you ask him to do. This coming winter we are going to prohibit them from taking beef cattle out, and, unless they show a disposition to help us, we are going to prohibit the railroads from receiving the cattle for shipment.

Mr. **Kittrell**. What is the extreme limit of the life of ticks without a host?

Doctor **Keane**. We usually figure on keeping the seed tick off the cattle for eight months, but I have kept ticks experimentally, and I have never seen them live over three months.

I believe in keeping the cattle off an entire year. There are other points in tick work. I have in mind one large ranch, comprising some hundred thousand acres, and the infected pasture comprises about 25,000 to 30,000 acres. We removed all the cattle, intending to keep them off until this coming January or February. They will feed on stubble, and thus we will keep the cattle off this infected pasture about eleven months. In California we also have districts where the ticks will live upon the cattle the whole year round, and other districts where they drop off.

Another thing we have been doing in California is to have practically everything arranged under an absolute quarantine. We enforce it, too, and the Government officials have been of great assistance to us in maintaining these quarantines. But the first thing to do in the eradication of ticks is to educate the people to the necessity of it, and then force them to clean up, and if you have not adequate laws you ought to get them.

Professor NEWELL. What is the average cost of dipping?

Doctor KEANE. In California we use a float dip. We use water with about an inch of oil on top, and we can dip cattle for about 4 or 5 cents a head.

Professor NEWELL. What does the oil cost for dipping?

Doctor KEANE. From 60 cents to $1 per barrel.

Doctor CURTICE. We have found a spray pump effective in applying oil in my district—in Virginia and North Carolina.

Professor MORGAN. I believe that after we have gotten together and talked over the general situation, and have mapped out distinct areas that may be different from other areas, we ought to have tests made of these various methods in connection with the conditions to which they are applicable. In my own case, for instance, we tried dipping; we tried washing; and when we struck the Beaumont oil and found that it made the temperature of the cattle go up we quit it. It almost meant the destruction of the animal, with a temperature of 100 and such humidity as we have there. Hence, when we developed this rotation method, it naturally appealed to most of us, and I concluded that it was applicable to the entire area. Coming to Tennessee, I found a different condition, and I think we are all broad enough to adopt the method that means tick eradication for the particular area in the easiest way. And I believe we should have definite experiments carried out in particular areas, if we can have this done through the Bureau.

Professor NEWELL. I think Professor Morgan has put his finger on the vital spot. We have got to study the conditions that we have to deal with in the different States; the remedy applicable in one will not be applicable in another. The gentleman from California [Doctor Keane] mentioned that the extreme life limit of the seed tick was about three months. Mr. Dougherty has said that seed ticks live for one hundred and seventy-four days. If you include the time required for the hatching of the egg after it has been dropped by the female, I figure that you have to add about twenty-five days, making a total of two hundred days. This is a striking instance of the difference between these different places.

THE LEGAL SIDE OF THE TICK-ERADICATION PROBLEM.

By GEORGE P. McCABE, Esq.,
Solicitor, Department of Agriculture.

Mr. CHAIRMAN AND GENTLEMEN: The organic act of the Bureau of Animal Industry provides that, whenever the plans and methods of the Secretary of Agriculture shall be accepted by any State or Territory in which pleuro-pneumonia or other contagious or infectious disease exists, or when such State or Territory shall have adopted plans and methods for the suppression and extirpation of such a disease, and such plans and methods shall be accepted by the Secretary of Agriculture, and whenever the governor or other properly constituted authorities signify their readiness to cooperate for the extirpation of any contagious, infectious, or communicable disease, then the Secretary of Agriculture is authorized to expend Federal appropriations in that State or Territory on investigations of the disease and on such disinfection and quarantine measures as may be necessary to prevent the spread of the disease from one State or Territory into another.

When the Department determined to take up the problem of tick eradication it was confronted with several vital legal and administrative questions. Under our form of government the eradication and control of diseases of live stock within a State are entirely within the police power of the State and are not subject to regulation by the Federal Government, except as may be necessary and incidental to the regulation of interstate commerce. It seemed that if the Department desired to expend a Federal appropriation on the work, and to use the organization of the Bureau of Animal Industry to help the States, it would be necessary that the inspectors and employees of the Bureau of Animal Industry should have legal authority to enforce such needful quarantine and disinfection regulations as might be agreed upon by the Department and the State. Moral suasion will sometimes accomplish wonders. For the general welfare of the community self-sacrificing citizens will often voluntarily subject themselves to loss and damage, but, as a general proposition, when it is desired to restrict citizens in the disposition and use of their property, a warrant for such action must be found on the statute books. Otherwise some obstreperous individual will kick over the traces and undo all the good that has been accomplished by the forbearance of his neighbors. Therefore, an examination was made of the statutes of the several States to ascertain the following points:

1. Are local officers authorized and empowered to enter premises to inspect live stock, to enforce the quarantine of counties, districts, farms, and ranches, and to control the movement of live stock therefrom?

2. Are local officers empowered to enforce such disinfection of animals and premises as may be necessary?

3. Are State officers authorized to issue rules and regulations establishing and maintaining quarantine lines?

4. May States confer authority upon Federal employees to act as officers of the State, to enter premises to inspect live stock, to enforce quarantine of counties, districts, farms, and ranches, and to control the movement of live stock therefrom, and to enforce such disinfection of animals and premises as may be agreed upon as necessary by the State authorities and the Department?

The search of the statutes disclosed several interesting facts. Four important southern States and one Territory had no statutes to cover these matters. Several others were prevented by the terms of the constitution from allowing any Federal employee to act in any capacity as an employee of the State. Other States were provided with adequate laws but no effective organization, while a few States had good laws and good administrative organizations.

I will not take up the time of this meeting by giving in detail the laws of the various States upon which the conclusions of the Department were based. In each case the conclusions were submitted to the Attorney-General of the State, and replies were received from a majority of those officials. I have the references to these laws and will be glad to discuss the matter individually with any of you. It may not be amiss, however, to state the general results of the examination.

At the time the examination was made, so far as could be learned, Alabama, Florida, Indian Territory, Mississippi, and South Carolina had no laws covering the subject.

In Arkansas the peace officers can enforce only such quarantine lines as are fixed by the State law or by the United States, and State officers can not issue rules and regulations establishing and maintaining quarantine lines, nor can they enforce the necessary disinfection of animals and premises.

In California local officers are authorized and empowered to enter premises, to inspect live stock, and to enforce quarantine, and are impliedly authorized to enforce disinfection of animals and premises. They are also authorized to issue rules and regulations establishing and maintaining quarantine lines; but the State can not confer authority upon Federal representatives to act as officials of the State.

In Georgia, Kentucky, Oklahoma, Tennessee, and Virginia the local officers possess all the authority necessary, and the State can confer necessary authority upon Government inspectors.

The law of North Carolina seems to be sufficient, with the exception that local officers are not empowered to enforce such disinfection of animals and premises as may be necessary.

While Missouri has laws on the subject, it is probable that local officers do not possess, in any particular, the necessary authority to carry on effective tick-eradication work. Missouri may not confer authority upon Federal representatives to act as officials of the State in such matters.

In Texas the local authorities possess sufficient power, but by constitutional amendment it is forbidden to confer authority upon Federal representatives to act as officials of the State in such matters.

Passing now from the examination of the statutes already in force to the consideration of an ideal statute for the work, not only of tick eradication, but for the control of all contagious and infectious diseases of live stock, the Department has, in response to requests from the officials of various States, indicated the essential provisions which, in the opinion of the Department, should be included in a contagious and infectious disease law. Briefly summarized, these provisions may be stated as follows:

1. The governor or other responsible State official should be authorized and directed, on the recommendation of a State board hereinafter provided for, to quarantine any portion of the State, no matter how large or how small, when he shall determine the fact that cattle or other live stock in such part of the State are infected with any contagious, infectious, or communicable disease, and to raise this quarantine at any time on the recommendation of the board. He should be directed to give proper notice of the establishment of quarantine to the officers of railroad, steamboat, or other transportation companies doing business in or through any quarantined part of the State, and to publish in such newspapers as he may deem necessary notice of the establishment of quarantine.

2. It should be provided that no railroad company, or the owner or master of any steam or sailing or other vessel or boat, should receive for transportation, or transport from the quarantined part of the State to a nonquarantined part of the State, any cattle or other live stock, except as will be hereafter indicated. All persons, companies, or corporations should be forbidden to deliver for such transportation to any railroad company, or to the master or owner of any boat or vessel, any cattle or other live stock, except as will be hereafter indicated; and all persons, companies, or corporations should be forbidden to drive on foot, or to cause to be driven on foot, or to transport in private conveyance, or to cause to be transported in private conveyance, from a quarantined part of the State to a nonquarantined part of the State, any cattle or other live stock, except as will be hereafter indicated.

3. Provision should be made for a State board to control the matter of quarantine, and this State board should be authorized and directed, when the public safety will permit, to make and promulgate

rules and regulations which will permit and govern the inspection, disinfection, certification, treatment, handling, and method and manner of delivery and shipment of cattle or other live stock from the quarantined part of the State to the nonquarantined part of the State; to enforce disinfection of premises and live stock, and also the purchase and destruction of dangerously infected live stock, and buildings liable to transmit infection. The board should be required to give notice of such rules and regulations in the manner provided for notice of the establishment of quarantine by the governor.

4. It should be provided that cattle or other live stock may be moved from a quarantined part of the State to a nonquarantined part of the State under and in compliance with the rules and regulations of the State board hereinbefore provided for, and it should be made unlawful to move, or to allow to be moved, any cattle or other live stock from the quarantined part of the State to the nonquarantined part of the State in manner or method or under conditions other than those prescribed by the published regulations of the State board.

5. Provision should be made for the appointment of inspectors to perform their work under the control and regulations of the State board. These inspectors should have the right to enter premises to inspect live stock, and to perform all the functions demanded by the law and the regulations of the State board made thereunder. These inspectors should perform their duties under the supervision of a competent State veterinarian.

6. Provision should be made for the payment of the cost of inspection, disinfection, quarantine, etc., preferably by a State appropriation, but if this be impracticable, then by fees to be determined and fixed by the State board and collected by that board. From this fund the salaries and expenses of the inspectors should be paid. The Department considers it bad administration to allow the direct collection of fees by inspectors as compensation for their services.

7. Provision should be made for the appraisal, condemnation, and remuneration to the owner for a part or the whole valuation of any premises or animals necessarily destroyed in controlling contagious or infectious diseases within the State.

8. The governor of the State should be authorized to cooperate with the Secretary of Agriculture in the prevention and eradication of contagious and infectious diseases of live stock, and, when the constitution of the State will permit, he should be authorized to appoint employees of the Department of Agriculture to act as State officers in contagious and infectious disease work. These employees of the Department of Agriculture should serve without compensation from the State, and when so appointed they should have all the power exercised by State inspectors. When the constitution of the State

will not permit such authority to be conferred upon employees of the Department, the work can be carried on by having a Department employee perform his work in company with a State inspector. However, this plan, in many cases, makes it burdensome upon the State, because it requires the employment of more State inspectors than would otherwise be necessary.

9. A suitable penalty should be provided for violation of the law or of the rules and regulations made thereunder.

If objection be made to the above provisions on the ground that thereby arbitrary absolute power is vested in the governor and State board, the answer is that, in the opinion of the Department, to secure effective control, prevent dissemination, and make possible the eradication of contagious and infectious diseases of live stock, it is necessary for this absolute and arbitrary power to be lodged in responsible public officers. The essential parts of the above provisions are, applied to State conditions, substantially those of the act of Congress of March 3, 1905, entitled "An act to enable the Secretary of Agriculture to establish and maintain quarantine districts," etc. To my personal knowledge there were no more enthusiastic advocates of the passage of the act of March 3, 1905, than the Senators and Representatives from the States you represent, and if the people of these Southern States are willing that this arbitrary and absolute power in regard to interstate commerce should be vested in the Secretary of Agriculture, as it is vested to-day, then no objection should be raised to the lodgment of the same power, so far as the State is concerned, in your own elective officers.

It is of course to be remembered that the above provisions may be entirely unsuited to certain local conditions, and the Department stands ready, upon the invitation of the proper officers of any State, to consider local conditions and to advise in a friendly way regarding the desirable provisions of a live-stock law for that State.

DISCUSSION.

Doctor LUCKEY. Did I understand you to say that the State of Missouri has not power to confer authority on the Federal inspectors?

Mr. McCABE. Missouri may not confer authority under existing law. There is no affirmative constitutional provision authorizing it to be done. The State authorities are not expressly authorized to confer authority upon the Federal officers.

Doctor CARY. I would like to ask a question in regard to the courts sustaining the actions of the board under the rules and regulations of the different States: Would the courts probably sustain the action of the sanitary boards in enforcing the rules and regulations?

Mr. McCabe. Under the constitution of a State, may a legislature fix a penalty for the violation of the rules and regulations of a sanitary board? Is that the question? So far as I have looked into it—I have not examined the constitutions of all the States on that point—the rule seems to be generally as in the Constitution of the United States. It is a constitutional axiom that the legislature can not delegate legislative power to any board. The legislature must do the lawmaking, but the legislature may provide that the board can exercise administrative discretion. The act of Congress of March 3, 1905, was drawn to meet that very objection. We have not found anyone who is willing to attack the constitutionality of that law. For instance, in Tennessee the State board will have the right to recommend that four counties be quarantined. Then as the law would stand it should be unlawful to move any cattle from those four counties in any way. But the State board of control may issue rules and regulations which shall govern the movement of cattle from those four counties, and any movement from those four counties differing from the manner prescribed by the State board would be unlawful. That makes it a violation to move the cattle except in accordance with those rules.

Doctor Parker. Is presence of the tick, *Boophilus annulatus*, special evidence of a communicable disease?

The Chairman. Doctor Melvin, can not you answer that question?

Doctor Melvin. If such a question should arise in court I think it would be the duty of the court to establish by expert opinion whether this tick was the disseminator of the disease, and I do not think there would be any trouble in convincing the court that this tick is the disseminator of the disease, just as infected clothing might be the disseminator of smallpox, or any other similar disease. Of course it would lie wholly with the court to construe it and to rule according to the expert testimony submitted.

Doctor Curtice. In North Carolina the *Boophilus annulatus* is mentioned in the quarantine law. That would be sufficient.

Doctor Melvin. I do not think it would be necessary to demonstrate to the court that any particular ticks were infectious ticks. The mere fact of this species being the conveyor of the disease, no other tick being the conveyor, would be sufficient evidence to justify quarantine.

The Chairman. It would be just like expert testimony in any other case. Would that apply to ticks on horses?

Doctor Melvin. Yes, sir.

Mr. McCabe. Under our Federal law, in case of a question as to whether or not an animal is infected, the burden of proof is always on the party who seeks to prove that it is not infected. That applies to healthy cattle as well as to diseased cattle.

Doctor PARKER. The Texas live-stock sanitary commission has taken the position that it can not secure the control of ticky horses, which, of course, simply embarrasses our work.

Doctor CURTICE. One point that comes up in the administration of the law is this: The laws are published in a book which is sent to the lawyers. The rules and regulations of the board of cattle control are published, but sometimes they are not sent to the lawyers; and the question comes up, What is proper publication? If they are sent to the lawyers only, the cattlemen do not get them. They may be sent to some cattlemen and not to others. Finally, they are changed from time to time, and those changes do not get into the hands of all concerned; thus the man that violates may not have an opportunity to get the law if the rules and regulations are part of the law; and it becomes very important to know by what method the States, and we ourselves, should inform the people of what the law is. It seems to me we should take more care in posting notices at the courthouses, and in posting weatherproof, untearable notices along the line.

The CHAIRMAN. We do that in Georgia.

Doctor CURTICE. Every time a change is made. These points we will have to consider, and they become more and more important as the lines moves in, because of the danger of the disinfected region becoming again infected.

Mr. BROWN. There is one point regarding our law in Virginia—and I did not observe that Mr. McCabe's paper covered it—and that is as to the power of the State board to authorize the board of supervisors of a county to quarantine against counties also in the quarantine district.

Mr. MCCABE. That was covered by the provision for the State board of control.

Doctor KLEIN. It has been my observation that considerable inconvenience is sometimes caused by quarantine regulations being put into force immediately—that is, they are not issued to take effect on and after a certain date, say thirty days after issuance; most of them become effective immediately. Changes are made in the quarantine regulations that cause great inconvenience and sometimes breed contention that would not exist if it were not for that fact. Of course I understand that it is not always possible to give notice; there may be cases where it is impossible to give notice; the situation may demand immediate quarantine. But I think there are many occasions where regulations are issued to cover a coming year, and we have learned by our experience of this year that certain departures from the present regulations would be required. In such cases, if notice of thirty or sixty days or three months were given of the new regulations, it would help matters considerably.

Mr. KITTRELL. I think one trouble in this State, and I presume in others, is that this law is not charged to the grand juries by the judge.

There are comparatively few people in the State who know there is a law on this subject, and it is not taken into cognizance by the juries.

The CHAIRMAN. Did you ask the judges to give it in charge?

Mr. KITTRELL. No, sir; but I believe it ought to be charged, and the grand jury ought to have power; and I think if that were done the law could be made very helpful and very effective.

Mr. MORRIS. I think these regulations are made to meet emergencies. When we find a county infected, it would be poor policy to give thirty days' notice. A man could take advantage of the opportunity to move his cattle. After the board has determined on rules and regulations the governor issues a proclamation that such and such shall be sufficient notice.

COOPERATION BETWEEN THE STATES AND THE FEDERAL GOVERNMENT.

By D. F. LUCKEY,
State Veterinarian of Missouri.

Mr. CHAIRMAN AND GENTLEMEN: No one doubts that in the work of the control of contagious diseases of all kinds, particularly in the work of controlling the spread of fever ticks, there ought to be the heartiest cooperation between the officers of the various States and of the Federal Government. We have come here to meet the officers of the Department of Agriculture, and I think this manifestation is a better proof of the spirit of cooperation which exists between the States and the Department than anything I could say.

After the enactment of suitable laws in the various States, then comes the execution of these laws. Each State and the Bureau should appoint competent, efficient, and industrious men and instruct them to work together. The line of cooperation that works in one State will sometimes not work in another. In the State of Missouri in the past we have failed to get results in some cases on account of the lack of cooperation and proper understanding between the officials of our State and those of the Department of Agriculture. A spirit of antagonism should not exist in any State, and I have done everything I possibly could to eradicate that before beginning the eradication of ticks.

A few years ago the regulations of the Federal Department and those of the State of Missouri regarding the handling of southern cattle were entirely at variance. The period of inspection was different, and the State regulations did not recognize a permit issued by a Federal inspector, neither did the Federal regulations recognize a permit issued by a State inspector. I have advocated that we work together closely, understand each other, talk candidly over things in which we differ, and have no misunderstanding; and I have advised the board of agriculture to make quarantine regulations, when they were necessary, to comply exactly with the quarantine regulations of the Bureau of Animal Industry. I am prepared to go still further, and, on the theory that the fewer quarantine regulations we have the better, I would recommend that our State withdraw her quarantine regulations altogether, as far as southern cattle fever is concerned.

I believe the protective line work should be wholly north of the quarantine line and should be left entirely with the Department of Agriculture, and that the cooperative enforcement of the line should be carried on where the line crosses a State. Such is not the case with the State of Missouri, and I do not believe there is a reason for the continuance of the quarantine regulations of the State. I believe we ought to have definite plans and that the plans and the work of the State officials and of the Federal Government should be in harmony. I also believe that whenever it is possible for those in the State to speak a good word for the work of the Department they should do so. I believe the officers of every State should do that.

DISCUSSION.

Doctor KEANE. A very important matter—the matter of a general live stock law—was brought up this morning by Mr. McCabe. I believe every State that is interested in this line of work should adopt a new live-stock law of the character indicated by Mr. McCabe. It will greatly facilitate this work, and I would like to hear a little discussion on that matter.

The CHAIRMAN. I would be very glad to request Mr. McCabe, with the approval of Doctor Melvin and Doctor Steddom, to prepare a general law, and I will guarantee, so far as Georgia is concerned, that we will amend our laws wherever it is necessary to conform to that general law.

Eight years ago we formed an association of the commissioners of agriculture of the Southern States. We found the fertilizer laws conflicting. Alabama had one law, Georgia another, and Tennessee still another, and they all conflicted as to the registration of the brands of fertilizers, the per cent of phosphoric acid, ammonia, and potash required, and the manner in which these goods should be branded; so that in Texas they had to have one brand on the sack, and in Georgia another. This was the condition we met in the different States, but after we had gotten together we undertook to have a general law passed by all the States in which fertilizers were used requiring that the guaranteed analysis, the manner of branding, and the way of registration should be about the same.

I think that same idea should get hold of us in tick-eradication work. We can not proceed on the best and most progressive lines unless we agree as to some plan like that mapped out this morning. I think Doctor Steddom and Doctor Melvin will bear me out in saying that we have a very good law for tick-eradication work in the State of Georgia. The whole business is left to the State department of agriculture and not to the governor. It is made the duty of the commissioner of agriculture to aid and cooperate in this work, and I think we have all the law that is found necessary. I have had the State attorney-general construe that law, and I asked him if amendments were needed. He said that the law was just as broad as was necessary. I talked to Doctor Payne about it and he said it was a good law. Doctor Klein thinks it is a good law. We formulated rules and regulations under that law. I hope some definite plan will be adopted by which we can all go home and go before the next legislature with a uniform law, and thus get together in this work.

THE ENFORCEMENT OF QUARANTINE REGULATIONS.

By THOMAS MORRIS,
Secretary Oklahoma Live-Stock Sanitary Commission.

Mr. CHAIRMAN AND GENTLEMEN: I suppose this subject means the enforcement by States. Oklahoma being a Territory and young, was fortunate enough to have an excellent quarantine law passed in 1897; but our resources being limited we were never allowed enough money to enforce it as it should be enforced. However, the legislature two years ago made provision for the appointment of seven inspectors, appropriating $10,000 for their pay, which, by the way, was not enough by $2,000 to employ them regularly. Since that time we have undertaken to give a more thorough enforcement of the Territorial laws. We made our rules and regulations, a proclamation was issued by the governor, and we began enforcing the law as best we could. Last year we began to make a range canvass in two counties. This year the work has been carried on by the Federal employees, and our own forces have all been engaged in it.

In enforcing these laws Oklahoma is peculiarly situated. Mr. Morgan speaks about disinfecting pastures by taking cattle off. Oklahoma being a new country, we find that will not do. A man breaks out a part of his land and leaves 40 to 80 acres for a permanent pasture. It may be all the land he has on his place that has a fence around it. That does not give him a chance to shift his cattle. When we find infection we quarantine it. We compel people to clean their cattle. Our law provides that an inspector may call to his aid the sheriff of any county to assist, but it is the policy of the board to give those people a chance. We instruct them the best we can, and if they will follow instructions they can clean their cattle without any expense to themselves; but if they do not believe in the tick theory we simply place those cattle in the hands of the sheriff and they are disinfected at the owner's expense. Our mode of disinfection is by greasing with crude oil. We do not bother with anything else. A great many dipping vats are being established over the Territory, and we encourage the establishment of them.

Our law provides that the Territorial board shall cooperate with the Federal authorities, and our rules and regulations must comply with their rules and regulations. That I think is a very good thing in any State law. Our board this year passed resolutions giving all Federal inspectors working in the Territory authority to enforce quarantine measures. The main thing in enforcing quarantine regulations in any State is to educate the people, to get them interested, to make them understand the importance and value of the work, to show them the depreciation of their cattle when they are below the quarantine line, and the advantages of an open market. Of course, we find a few people who will not listen to reason. Those people we simply show.

DISCUSSION.

Doctor **Keane**. I consider maintaining the quarantine a very important feature in this work. We sometimes overlook the fact that we ought to place individual quarantines in the quarantined areas; that is, a range should be kept in quarantine. The only way to do this is by proper live-stock laws and proper enforcement.

Mr. **Morris**. That has been our work in Oklahoma during the last two years. We make a regular inspection. We inspect all the pastures about this time of year, and if there is no infection we release the pasture until next April, holding it in quarantine for later inspection. Since the tick-eradication work has been begun and we have the help of Federal inspectors, we do not need to do that. If a man applies to us to move cattle and if they are inspected and found clean we allow him to move them. He can move no other cattle. We hope for great results, and there is every indication of our hopes being realized; but the individual quarantine is the main feature in ridding the country of ticks. When quarantine is placed there is a notice, and the man is instructed by our inspector. The cattle must be thoroughly greased every thirty days, and we generally give a lesson in greasing. As I said this morning, our men are all cowboys and can rope a steer. Their plan is simply to throw a rope over his horns and heels so they can throw him down and grease him all over. I think that the dipping vat is the proper way, but it is expensive, and the farmers can not all establish vats; but they are establishing them in neighborhoods, and we have adopted the dipping plan wherever they have a dipping vat in the neighborhood. We allow all cattle in the neighborhood to go to that vat, but the work must be under supervision. The cattle must be under supervision before leaving the pasture, to prevent infesting the public highways. We have an inspector present at the dipping. The people have peculiar ideas.

Men in the eradication work have quarantined pastures, and probably afterwards received a letter from the owner stating that his cattle are clean and asking that they be released. The idea has been for the inspector to follow up this work twenty days after the first inspection. We have not been able to do that in all cases, but we are doing it now. We have a force in hand so that, whenever a man applies for inspection, we can furnish a man to visit his pasture, and if his cattle are free they are let out.

Mr. KITTRELL. Does your law compel a man to clear his own place from ticks? I know you can quarantine them.

Mr. MORRIS. The law says we can enforce such local measures as are necessary to get clear of the infection, and I believe we have the power to make them do it. If a man will not, we put the cattle in the hands of the sheriff. When we quarantine cattle, we take them out of the owner's hands and turn them over to the sheriff, the same as if levied on, because he can levy and sell at the owner's expense if he does not pay.

Professor NEWELL. How do they enforce the regulation?

Mr. MORRIS. By the penalty attached to our law, the lowest fine being $100.

Professor NEWELL. Well, you must get the evidence to convict them. A man may move his cattle away overnight.

Mr. MORRIS. Some of our judges have not given us a fair show. We did get a decision in the district court of Noble County. The man's farm is quarantined; the line is around his farm. The judge held that, where it could be proved that cattle were on one side of the line one day and on the other side of the line at another period, that constituted a violation of the quarantine law.

Professor NEWELL. But you can not prove the identity of the cattle except by branding.

Mr. MORRIS. We do not find any difficulty in that case. In our county the people who haven't got the ticks are very particular about reporting violations.

Professor NEWELL. Have you ever worked in a locality where a man will protect his neighbor?

Mr. MORRIS. About one out of ten is antagonistic in our locality.

Professor NEWELL. I don't think men would obey a law providing for individual quarantine in Louisiana. We can not depend on compliance with such a law, because it would be considered absurd.

Mr. MORRIS. I have so far had only about three reports of persons who have moved their cattle from quarantined pastures before we released them; I have inspectors working on the evidence, and those men will be prosecuted. We never fail to stick a man for his $100 fine.

EDUCATION AND PUBLICITY AS AN AID TO TICK ERADICATION.

By C. A. Cary,

Professor of Veterinary Science, Alabama Agricultural and Mechanical College.

Mr. Chairman and Gentlemen: I do not represent one of the leading States in this work, but I do represent a State that is pretty well infested by ticks. Doctor Steddom reported that a majority of the farms inspected are infested. My plan of work has been to use the farmers' institutes to educate the farmer. Out of 85 sessions last year—from the 1st of July, 1905, to the 1st of July, 1906—I do not think we had a session at which the tick question was not discussed thoroughly, if not by myself, by Professor Morgan or someone else thoroughly conversant with the subject. When we commenced the work this fall in the two counties bordering on Tennessee we began to write on the subject. We kept the county papers more or less full of the tick question for several weeks. This year men went up along the line between Tennessee and Alabama and had township meetings of all the farmers we could get out in those townships. In addition, we thoroughly discussed the question with individual farmers. I said to the inspectors: "When you get to a man's farm tell him all you can, and look over his farm and see if there is any particular advantage in using this or that method, and discuss it thoroughly with him." I do not think we should take just one method and go out and harp on that method and nothing else, but if we see that one man can readily starve out the ticks, give him that method. If he has but a few cattle—as some of the negroes have—he can clean them by hand. We advise them to grease the animals, and we keep them at it. Then I hold out to them that if they do their part by a certain time we will try to get them out of quarantine. Of course we do not promise to get them out. In this work we have had no law at all—not one word of a law. Once in a while we run across a man who is opposed to our work, and we say, "How many cattle have you?" "That is none of your business." We go on by and find out from his neighbors something about him. We are going to get a law this winter.

Now, I want to say a few words to the inspectors, without meaning any offense whatever. Inspectors sometimes do not know some things they ought to know. They ought to know more about the cattle-farming conditions in the communities in which they are inspecting. They know many things about the life history of the tick and about veterinary science; but if the farmer should ask some simple practical question and the inspector answers "I don't know," what does the farmer say? He says, "That fellow is not up to his business." I know this from experience. I find that the inspectors coming into Alabama ought to have a course in Alabama agriculture along the lines of rotation and diversification of crops.

You can not make a general rule. You must say "Have this crop here and that pasture there." When the inspector comes up against a farmer, he should have a practical answer right on the end of his tongue.

Let me tell you now where some of you are going to run up against a difficulty next spring. You have told the old farmer that if he will grease his cattle he will be rid of the ticks. Along next spring the ticks' eggs will hatch out, and his cattle will be reinfested. Then he will say "You lied to me then, and I don't believe you." We may just as well face this thing. Be careful about what you promise these old farmers, and when you promise to do a certain thing do it in such a way that it will bring success.

I wish every inspector that goes into the South, especially from the North, could go to some of these short-course agricultural schools and learn the agricultural conditions of the South, so that he will know what to do. You ought to learn the main principles in a very short time if you go to one of these places where you will be properly instructed. We have a ten-day course in which we have addresses by the superintendents from different States. We have from 500 to 600 farmers in attendance, and we give them instruction. If the inspectors could have the benefits of such a course, they would be better prepared to tell the farmers what to do, and their advice would not be too general. The farmer wants you to say just what to do, and he wants to realize that you know what you are talking about. Then he will believe you and follow your advice, and will do a great deal more than if he thinks you are telling him something that is not true.

If you go into a township and tell all you know about the ticks, you may have to repeat and repeat in order to get it into the men's minds. But you should recollect that the more you get it into one man's mind the more he will spread it among the others. In Alabama I am often asked to make a "tick talk." They laugh when I get up, and say, "There comes the tick man again." Let them laugh. I never let an occasion go by without having the tick question discussed.

DISCUSSION.

Mr. Brown. I have in Virginia been a member of the board of control since the matter was turned over to the State, or rather since the line was run through the State. This question of bringing the matter home to the people is the most important thing in the work of tick eradication. You must bring it home to the people who own the cattle. In the last few years we have been trying to get the counties along the quarantine line to take enough interest in the matter to appoint local inspectors and support them. Our State supports the quarantine work and pays all the expenses of such officers as we send into the field; but under our constitution no money can be paid out of the treasury except in pursuance of appropriations, and the work has grown faster than the appropriations. We have now at work in Virginia over fifty local inspectors who are acquainted with the farming conditions in these counties. It has been our good fortune to have the cooperation of the United States authorities. I believe Doctor Curtice will bear me out in stating that the work of the local men has been good, so much so that Doctor Curtice and our State inspector reported this fall to our board that ten counties had been freed from the cattle tick, and recommended that we release those counties from quarantine and leave in quarantine only such farms as were affected. That recommendation was approved by the Secretary of Agriculture on December 1, and ten counties in Virginia were released from quarantine.

When you can get the people interested you have a decided advantage in the educational work. Of course the local workers are not college graduates, and possibly they are not as highly instructed in the life history of the tick as they should be, but when their work is coupled with that of the Bureau inspectors we have an ideal working force. With the United States Government and the State government cooperating in the work, the State and the counties furnishing local men who are there all the time, it strikes me that very decided results can be accomplished.

We have simply used the starving-out process in Virginia. We have recommended to the people of the counties the "no-fence" law, under which each man fences in his own cattle. The fence law in Virginia is left in the hands of the local authorities. Any county can adopt a "no-fence" law for that county.

I do not believe in going too fast, but I hope the work done this fall in Virginia by the Federal inspectors will bear good fruit, and I believe that is the true line of publicity.

Professor WILLOUGHBY. The educational side of tick eradication is very important. In fact, it is generally recognized as the first thing that must be done in our work, and upon the method of this first work depends the success of the work that is to follow. This work should be done carefully, and it should be done as enthusiastically as possible; yet we should not present before any audience the idea that tick eradication will be easy, because they will find out later that it is not so. Still I believe we should present these matters in as favorable a light as possible.

I hope that the future appropriations given to the Bureau will make it possible for more inspectors to be sent to the farmers' conventions, to the meetings of the live-stock associations and agricultural societies, and even to the regular farmers' institute meetings, as they may be held in the different States. While most of these inspectors are quite busy superintending the details of their work, it occurs to me that perhaps other men who are gifted with the power of oratory might be detailed from the Bureau to go into different States at different times of the year and present the question of tick eradication. Another good plan would be for all inspectors engaged in this educational work to use the local newspapers more than has been done in the past. Of course the agricultural papers should be used to the fullest extent. In other words, keep the work constantly before the people until their minds become so imbued with it that they realize it is a good work and will constantly support it.

I also hope that at another meeting, such as this, we may be able to have more of the local inspectors present. It would be a great source of enthusiasm and inspiration to the men who are out on the firing line.

Mr. A. H. PETTIBONE. We have in Tennessee a most admirable law, but it lacks just this—it is printed in the acts of the legislature and a few copies are sent to the county clerks, and there it stops. The lawyers and the people know very little about it. The law ought to contain some provision making it the duty of all the judges in the State to give that law in charge to the grand juries. I found in my investigations over the State of Tennessee that the law is not known to one citizen in five. I want to get the law to the people. You can not enforce such a law unless you have cooperation between the Bureau of Animal Industry and the various authorities of the States. There should be the most cordial cooperation, and I am thankful there is in Tennessee.

I have published in forty-seven different newspapers in the counties that I have visited a two-column letter, and I have not yet found an editor who was not willing to publish my letter. These letters have gone right into the farmers' hands. Thirty-seven of the State papers have published my letter on the cause and the cure of the Texas cattle fever, urging always the cooperation of the people with the authorities in the enforcement of the quarantine. I have gone to the county boards, to the lawyers, to the editors, and in that way endeavored to get the cooperation of the people; and I find the people are perfectly willing, but they are uninstructed; they do not know the law. Who will go to the county court clerk and get a copy of the State statutes, hunt up chapter 156, and read it for his information or his direction?

In a recent trial in the county of Carroll a man whom we had to prosecute was defended by a young lawyer who did not know of the existence of this act of 1901. Here was a lawyer of high intelligence, a man elected to the next legislature, who absolutely did not know of the existence of the law. What, then, can you expect of the common people?

The **Chairman**. In Georgia we have our laws and rules and regulations relating to this matter published, and we send out ten or fifteen thousand copies.

Mr. **Pettibone**. That ought to be done in Tennessee. I think a compilation of that kind ought to be made right away. The best document that I have yet seen on the subject is Circular 97 of the Bureau of Animal Industry, "How to Get Rid of Cattle Ticks." In my work I have distributed many copies of this circular by mail and otherwise.

Doctor **Parker**. I wish to be recognized by the chair this time as representing the live-stock commission of Texas. Texas has had troubles different from most of the other States represented here. Cattle in most of the States are a kind of "side line" of agriculture. Over a large part of Texas and parts of California we have ranches where they raise nothing but cattle, and the profits of the business depend on the ability of the people to move their cattle to market. One of the greatest educational methods we have had there is old Mother Nature. The losses from splenetic fever are a powerful educator. A good many of the ranchers have been unable to move their cattle when they wanted to without violation of the law; they have been hampered. This, also, has educated them. With the proposed strict enforcement against the movement of ticky cattle above the quarantine line, which we are beginning in Texas in all localities, there will be some more education.

Most of our inspectors in Texas are trained in tick work and understand the disease; they also understand ranch work, and can talk intelligently to the ranchman about the best management for his own ranch. A majority of the men at work both for the Bureau and for the State of Texas can go to a ranch and in a short while understand the conditions, and can tell the ranchman what he must do. The inspectors are posted on rotation of crops. There was a ranch of more than 150,000 acres, more or less ticky, above the quarantine line. The owner had read the papers and knew the law on ticky cattle. He also knew more or less of the dangers of splenetic fever; but, with all his knowledge and experience, he was not up to the point of action on the eradication of ticks. It was only when one of our inspectors had a personal talk with him and explained things to him that he became convinced that it was money to him to eradicate ticks, and he said to his ranch foreman: "Put in as many dipping vats as are necessary, improve the fences, and eradicate the ticks next year. My land is worth $3 more an acre free of ticks than it is when ticky." That kind of education pays.

I believe in the use of newspapers. So far I have made only one talk on this subject, and that was out in a cattle country. We were there handling the open-range proposition—extermination of the ticks on the open range. That is a hard problem—five or six hundred square miles with not a fence. I gave them an opportunity to ask questions, and after the meeting closed they still kept on asking questions. I have a pocket full of letters saying this man and that man is interested in this work and has pledged himself that he will dip his cattle; that he will follow the plan we outline. That is good educational work, but it will take time to secure results in that kind of locality.

Doctor MELVIN. With reference to this farm-management problem, I should like to ask Doctor Cary whether it would not be feasible to have special meetings for inspectors. Of course they should not all be held in one place. They would have to be held at several points. Would it not be feasible to have those who are versed in farm management give instruction to our inspectors? What is the length of time such a course would consume?

Doctor CARY. If the inspectors in one or two States could be gotten together for two or three days, and could have a course along the line of rotation of crops, it could be done very cheaply. Most of the lecturers could be gotten there for their expenses. I think it would be a very wise thing to have such a meeting once a year. I believe Georgia and Alabama could be combined. It would occupy two or three days, provided the men have already had some drill along these lines. I hope Doctor Melvin can see his way clear to holding some such meetings. I believe the average man could get a good outline in three days, with three lectures in the morning, three in the afternoon, and one at night. It might even be done in less time.

My experience has taught me the great value of personal contact and instruction. We have some 15,000 names on our experiment station list, and every time a bulletin is issued we mail a copy to each address; but when I go around over the State with the station superintendent to lecture on the same subjects I find that about nine out of ten of these men have shoved these bulletins into pigeonholes, and that is the end of them—they do not read them. The same is true of this work. But when we get out and talk and let them ask questions we can convince them as we can in no other way. This way of educating these men beats all the writing in newspapers or sending out bulletins.

Doctor LINCOLN. In Tennessee we do not have any trouble in getting in line the farmer who has a number of cattle. Here 25 cattle are considered quite a bunch. That owner is easily reached by talking the financial considerations to him. But we do have trouble with those who own only 1 or 2 milch cows. If there were some way of handling that class of farmers the proposition would be much easier.

The CHAIRMAN. It is the same way in Georgia.

Doctor LINCOLN. I would like to know how to handle that class of men. They have nothing to sell, and do not expect to have.

Doctor CARY. When I find a man like that I quarantine his cattle. I think we need laws in the different States by which we can compel men to clean up their farms.

Doctor CURTICE. The greatest educational force has been the restraint of the cattle trade. First men learned that they did not want cattle coming from a certain direction to pass through their neighborhood, because it caused their own cattle to die. So they kept the cattle back, ticks and all, and that was education. Following upon that a quarantine line was established from Virginia to California, and the quarantine has increased in severity from the time it was first established until now; but even yet it does not seem to be thoroughly effective. There are ways and ways of marketing cattle, and a man who has quarantined cattle thinks that it does not matter to him whether his cattle are ticky or not. I think, of course, being an inspector, that we should use the strong arm of the law as the main factor; I believe we should ever look to increasing the severity of quarantine against the infected animal. The placing of quarantine upon areas instead of counties has been a move in that direction. This educates two classes of men—those who own the cattle, and their neighbors. You are told many times that "it does not matter to me," but the man who says that forgets. Next day he is running to you—"I would like to take that cow over to my neighbor's." If we have good quarantine service it is then that we get in our work. We will have the single-cow man working better than the man with a dozen cows, who intends to market them November 1, because he can get the ticks off by that time. He has been driving his ticky cattle off after November 1 or December 15, as the law may read. If the States will support us there is going to be no driving of the ticky cattle, unless the owner has done something for them. We have tried to bring that matter before the people of Virginia, North Carolina, and Alabama. I believe even now that the people are waking up to the idea that their cattle may be in quarantine next year.

I am talking about the strong-arm work in education. Some people will not or can not read the county papers. One of the recent violations was by a man who could not read nor write his name. He depended on others. He was told the quarantine expired November 1, when the proper date was December 15. It must be our part as inspectors to know how to get the information to every man who has a cow. We must put a big red poster upon the barn when a place is quarantined. We must put posters upon the quarantine line, in the court-house, and in every suitable place. Publication is cheap as compared to delay in enforcement of the law.

INTERSTATE LIVE-STOCK ASSOCIATION.

Doctor LUCKEY. I wish to take advantage of this occasion to announce the meeting of the Interstate Live-stock Association at Jamestown, Va., some time in the latter part of next summer. I think that if this meeting here to-day has demonstrated anything it is the fact that the officers of the various States are too far apart—working in too many directions—and I believe that the association I mentioned should be made a permanent one; it should be attended by representatives of all the States, and should be taken as authority on matters of sanitary legislation and on contagious diseases. I promise you that I will help to arrange a program for the next meeting that will be of especial interest to all the States. I want to make this suggestion. There are thirteen States interested in the extermination of ticks; there are other States not interested at all, but some of them are interested in breeding cattle and in the subject of tuberculosis. There is going to be a lot of money expended in controlling tuberculosis, and those who expect the northern people to help pay for the extermination of ticks will in return have to help the northern people pay for eradicating tuberculosis. I hope that you will all attend the meeting at Jamestown in 1907.

REPORTS FROM STATES.

ALABAMA.

Doctor CARY. This year the work has been extremely hard. The Bureau has furnished the entire amount of money for that work. I was promised by the State authorities, before Congress appropriated the money to cooperate with the different States, that they would authorize a like amount, but when I put the question up to them they refused. Doctor Melvin took up the work, and we started in two counties. We had no law, and what we have done has been largely along the line of education, in connection with the farmers' institute work. With one inspector in each county, and with what work I could do, we have accomplished something in the line of education.

ARKANSAS.

Doctor LENTON. The work has been a study of existing conditions rather than aggressive measures in tick eradication. If we can get a good idea of the conditions affecting this subject, we shall then be able to take effective measures at a later date. The inspectors in Benton County unfortunately could not get over the whole county. About two-thirds of it was inspected. All told, 77,474 cattle were inspected; 75,993 were clean and 1,481 ticky. This gives a little less than 2 per cent of ticky cattle in the northwestern county of Arkansas.

Probably this county, being next to Indian Territory, gives a far worse showing than the counties east of that in the northern tier of counties. Probably there will be some counties in the northern tier which are completely free of *Boophilus annulatus*. In Washington County the work was begun too late to form any definite idea of the amount of tick infestation there. The number of cattle inspected was 2,003, with 903 ticky—45 per cent. I do not think this is a very fair indication, as only four townships were gone over, and those near Indian Territory. The greater part of the infection in northern Arkansas comes from the Indian Territory. Other small centers of infestation are due to shipments of cattle by rail from the Territory to the States through Arkansas, as for instance where the Kansas City Southern crosses the Frisco line. There cattle are unloaded from one system for reshipment on the other. Thus small centers of tick infestation are created.

It seems to me that it would be a very great incentive to work in tick eradication if our northern tier of counties were allowed inspection all the year round. Possibly excepting Sharp County the whole northern tier of counties might very well be open for inspection the year round.

FLORIDA.

Doctor Dawson. As I said a while ago, Florida has not done anything in regard to tick eradication. We look upon the tick as a necessary evil. All the work which has been done has been along the lines of education—publication of bulletins and inoculation of cattle against Texas fever. We have quite a number of men who know all about ticks and the trouble they cause, the loss to cattle in flesh and milk, and so on; but the State is not ready to take up the question of eradicating the tick. At least there has been nothing done yet, and you know how long it takes to do the preliminary work of eradication. However, there is no good reason why it should not be done. This is the first public recognition Florida has made of the cattle tick, and I think it would have a big bearing upon the question of taking up tick eradication if I can get a good report of this meeting before the board.

We have powers of quarantine. I frequently quarantine animals, but I have exercised the power only against hydrophobia and glanders. We have not the power to destroy any animal, but we can quarantine them, which is nearly as effective. The right to quarantine has never been questioned. We just put it in the hands of a deputy sheriff, and if he does not enforce it we have a man who will. Florida has rigid laws in regard to public health, and the people are more or less expectant of things happening to them in the way of disease. When the State board of health was first established, being a new thing, there was more or less shotgun talk in the State in regard to the enforcement of the laws, and the people have learned to respect our public health laws; and all the laws concerning the diseases of animals are embodied in the laws of the State governing the public health. I hope at a future meeting that I may be able to report something which has been done.

GEORGIA.

Doctor **Payne**. The work in Georgia did not commence until late in the season, the first active work being done the last week in September. The force consisted of four Bureau veterinarians and ten agents appointed from the different counties in the State. The national inspectors worked through cooperation with the State inspectors. The work was started in the mountainous counties in the northern part of the State, and, while the results of our work were not as satisfactory as we had hoped at the start, I think we have really accomplished some good. The seven counties in which the work was started are adjoining the three which are now outside of the modified quarantined area. They are hard counties to work in. Four of the counties have no stock laws of any kind whatsoever, so we practically have the same condition of affairs as they have in Texas or California, or any other open-range territory. We have an open-range country, and what makes it worse is the fact that the cattle must be taken off that open range and kept in close confinement during part of the year. Some might think that is a good time to rid the cattle of ticks, but on account of mild climatic conditions in Georgia it has been positively ascertained that the ticks are carried through the winter in sheds and other protected places. In other words, the infestation is kept in the protected places during the winter and is carried back to the open range in the summer. From the best knowledge I have I do not believe that the infestation remains on the open range in the high pastures in the State of Georgia. From the first of October or the middle of November until the first of April the next spring the cattle are not allowed to go on the open range. Infestation does not show itself in the spring, nor until along in the middle of summer. There are a few cattle kept in protected places that have ticks on them the entire year, and they have at least one crop of ticks during the winter season. For that reason we have a rather hard condition to combat in Georgia.

My views on a number of things are somewhat opposite to statements which have been made here to-day. But perhaps somebody can enlighten us, so we can better combat the conditions which prevail in my State. There has been a great deal said about education. Education to a certain extent is undeniably a good thing, but you may call upon a stock owner and find his cattle infested, and quarantine them, and spend all the time that you think is necessary to enlighten him, to the best of your knowledge, on every point. Return to his premises in a week or ten days, and you will find that no effort whatever has been made to follow the instructions. You may talk to him again, and come back in two or three weeks more and find the same condition of affairs. After you have called upon this stock owner three, four, or five times, I think the time has come to stop your education and pursue some stricter measure. That is the state of affairs in a number of counties in our State. I am glad to be able to say that in some of the other counties, where we have a better class of citizens, we find it easier. In three counties we have stock laws, or what are known as no-fence laws; that is, there are no fences around the crops; but even in those counties the cattle are found at times on the highways. We have accomplished considerable good in those three counties, and I believe that if the work is carried on and a close supervision maintained up to the first of July or August at the outside, we can eliminate the tick from those three counties.

Doctor CARY. I would like to ask what percentage of men he finds who will not do anything after these visits.

Doctor PAYNE. We can not apply the rotation of pastures in those open-range counties, consequently that does away with the rotation of pastures. I can safely say that in the four counties with 15,000 cattle in which the cattle owners have been instructed to pick and grease their cattle, there are not 100 cattle that have been thoroughly cleaned. It has been stated here to-day that that is one way to eradicate ticks—to thoroughly pick and grease—with which I concur. But I do not find one man out of a hundred who will thoroughly grease or pick the cattle. On reinspections, where we expect to get the best results, we find that prominent men, whom we expect to assist us in the work, have not picked their cattle clean and the greasing has not been thorough.

KENTUCKY.

Doctor EISENMAN. Geographically Kentucky is so situated that if Tennessee would take care of itself Kentucky would get the benefit. In former years we had a good deal of Texas fever, this being due to the irregular handling of cattle at the Bourbon Stock Yards, but since we have had a corps of efficient inspectors at the yards we have had no recurrence of Texas fever, with the exception of last year, when I think there were in all three cases, the infection being introduced from the slaughterhouse.

Kentucky has the best of laws, I believe, for the control of infectious diseases in the human family and in cattle. They are operated under the immediate direction of the State board of health, and the work is done effectively. The counties of Clinton, Wayne, and Pulaski have been in quarantine for five or six years, but I never had the pleasure of making a visit to those counties, for I could never get the county officials to recommend that there was anything there requiring my services. In cases of that kind we fall back on the Bureau of Animal Industry. We are very willing to delegate the authority to exterminate the tick.

Personally, I am of the opinion that this body should go on record as favoring some suitable laws, such as Mr. McCabe suggested—something that will be uniform and, as far as possible, apply to all the tick territory. Whenever the cattlemen or some few of us who are interested in this work propose anything, we always meet with the same old cry—"There is somebody who wants a job." That kind of opposition is very hard to get over. We were successful in enacting a glanders law about five years ago, because fortunately the infection appeared at a place right in the immediate neighborhood of the best race-horse farms in the country, and of course that brought the best people to the legislature, which was in session at the time, and in less than twenty-four hours we had a very suitable law. I do not know of anything that will operate so effectively to get proper State laws and appropriations commensurate with the amount the Bureau of Animal Industry is willing to expend as to make it known to the people that it is not a local matter but a national one and that it is absolutely necessary to have their cooperation.

I would ask Doctor Ellenberger to give us a synopsis of his report on certain counties. The State veterinarian, whenever an application is made to look up a suspicious case, is very politely informed that there is nothing there and his services are not required.

Doctor **Ellenberger**. In reference to the work in Kentucky, we first started by meeting the boards of health of Wayne, Clinton, and Pulaski counties. Pulaski County is really free of infection, but a little corner of it was left below the line simply for a shipping point from the infected territory of Wayne and Clinton counties, as there had to be some way out for these southern cattle. We started in the work by visiting these counties and meeting the county boards of health and as many stockmen as we could get together, simply to find out if they were willing to get together this year. The county boards of health were not particularly interested, and they were not willing to go ahead unless urged, so we got the principal stockmen to urge the county boards of health to take up the work. It took considerable time, and it was well along in August before we got Wayne County interested. In Clinton County they commenced at once by appointing one man to look over the county. By the time the Bureau got ready to organize, the county had appointed the magistrate of each precinct to take up the work as an inspector, and the Bureau put in as many men as were considered necessary. We were very much surprised to find that the northern part of Clinton County was generally infected, and that the infection had extended into Russell and also Cumberland. That required more help, and we put more men in there than we at first thought necessary. But the counties came to the front readily, and Wayne County had two men on from the time the work was begun and four men part of the time. Clinton County had on five men a portion of the time and then cut down the number to two men—that is, in addition to the work the Bureau did. There was no trouble in getting Cumberland County interested, and that county employed two inspectors. In Russell County there was a very small section infected. Out of 37 farms in that section 7 were found to be infected or exposed. The cattle were running at large in that neighborhood. The inspectors reported that on reinspecting these farms as many as three or four times no ticks were found. I think we have accomplished a great deal. We have done better work in those counties than in similar territory in Tennessee. The county boards of health came to the front and cooperated very well.

LOUISIANA.

Professor Newell. Louisiana has no live-stock sanitary law under which the diseases of live stock can be controlled with certainty. We have not only Texas fever to contend with, but also charbon or anthrax. However, we have a crop pest law which gives the crop pest commission the power to make and enforce regulations to prevent the spread or introduction of crop pests. Our best lawyers, district attorneys, and the governor (who is ex-State attorney) construed that law in its broadest sense—that cattle are a crop; therefore a tick must be a crop pest. Our crop pest commission, therefore, can make regulations dealing with this as well as any other crop pest; and the statute that created that crop pest commission gave it the power to make all necessary regulations and do all things necessary to prevent the moving of any and all things whenever such movement might cause the spread of any crop pest. We can take hold of the tick with these regulations and incidentally we shall have taken hold of Texas fever.

In Louisiana I think we have undoubtedly the most heavily infested portion of the United States. You people, who are working along the quarantine line and in the mountain counties only, hardly know what tick infestation is. We have not established very many dipping vats in Louisiana, but nearly all we have established are used in tick work. The work was commenced along in 1897 or 1898, when Professor Morgan realized that we had conditions in Louisiana, with our humid climate, where the life history of the tick differed from what investigators found elsewhere. Professors Morgan and Dalrymple met with a very favorable reception on the part of the people. Bulletins Nos. 82 and 84 of the experiment station were issued last year and have already been exhausted. At the present time we are getting a great many requests from the people for tick literature. We realize that the work must be educational. We can not go against public sentiment, and we are slowly getting the people to understand that here is one of the greatest problems that has ever affected the prosperity of Louisiana agriculture. We now have in different parts of the State men who are undertaking the eradication of ticks upon their own farms. Among the obstacles we have to contend with, perhaps the greatest is the objection brought forward by the man who eradicates the tick from his premises while his neighbors do not do likewise. We have no quarantine line to push south or west or east. We have to start somewhere, and, after establishing a small tick-free area, enlarge it. The question is to find the point and make the start. Even if we start on one man's farm he brings up this argument: "I do not want to free my cattle. Some one else's cattle will break in here and I will get a bad case of fever. I would rather keep down the ticks." This is one of the hardest things we have to overcome. We have the free range practically everywhere, and we have got to bring the people to realize that the free range must go. We have conditions in different parts of the State which vary greatly. We have rice, we have sugar-cane, and we have great cotton fields; then we have the pine lands and the uplands. In north Louisiana you can find almost as many different methods of farming as in all the rest of the South.

Our crop pest commission has been doing work in cooperation with the experiment stations for something over a year. Our commission has spent already in the neighborhood of $2,000, principally along the lines of life history and biology, and we have a few thousand dollars more which we expect to expend in this way; and we expect to get a good appropriation from the next legislature. Our work in Louisiana, I am glad to say, has been received very favorably, and tick eradication strikes the people as something worth looking into. Doctor Curtice has accused me of being a pessimist, but we are not pessimists in Louisiana. At the same time we do not want to underestimate the magnitude of the work which we have before us; and while we shall work faithfully and as long as we have anything to work with, yet for my part I expect to see Louisiana the last stronghold which will have to be taken by this army of exterminators; but by that time I think we shall have weakened the forces of the tick somewhat in that State.

NORTH CAROLINA.

Doctor **Curtice**. In the two States that I have represented (Virginia and North Carolina) the work has been conducted very differently. We began very early in North Carolina; Doctor Butler was already doing excellent work in that State, and it seemed to be a good plan, at least for this year, for the Government inspectors to overlook the work which had already been done in part. Five counties—Polk, Mecklenburg, Cabarrus, Davidson, and Forsyth—will, I think, be released from quarantine, and parts of McDowell and Rowan. Doctor Butler's system has been in the past not to release a county before he had confidence that not more than 12 herds would be left infected in that county for quarantine work the next year. He has merely adhered to the same rule in this instance, with the exceptions that in Rowan there may be about 20 herds next year, and in Davidson there are now 30 in one locality, but they will be reduced to about half. He has four other counties—Yadkin, Rutherford, Cleveland, and McDowell—on which he has been at work two or three years; and I think that Rutherford will not have 20 herds infected next year, Cleveland will have 30 to 40, McDowell perhaps as many, and Yadkin, which had 100, may have about 5. No new territory has been taken up by him this year. He has held out three counties—Anson, Union, and Stanly, east of the Catawba River—for work next year, and seven or eight on the northern boundary of the State contiguous to the area, which must be worked with Virginia.

The main part of the work in North Carolina has been done through local inspectors by Doctor Butler. Our men have worked there two months and a half and have made good progress, but, as Doctor Butler says, you can not do it in a season. There will be less infection next year, but the main part of the work is coming then. We have not gone into the counties in that State with any idea that the work of one season would educate the men or completely clear the areas. Doctor Butler's difficulty has been for some years in not finding enough infection in some of the northern counties. You have to hunt close to find the infection. For example, in Forsyth there were 3 herds in the southern part recently infected. I believe that if we had proceeded a month earlier in Forsyth County we might not have found infection in the southern part. It had been driven in from counties that were not quarantined against—Stokes on the north, in the group of counties in which he was working. The two tiers of counties to the east of Guilford are about in the same condition. We may or may not find infection in those counties, as it has been gradually decreasing.

OKLAHOMA.

Mr. Morris. The Bureau in its regulations last year provided for the movement of cattle from most of the counties in which we have made investigations this year. The Territory never permitted the movement of cattle out of those counties under those regulations simply from the fact that they are so much infested that we did not think it was safe; but the coming year we have asked for the same regulations. We think we have all the herds under quarantine and such herds will be safe the coming year. When we make an investigation of a part of a county, locate infection and quarantine it, we immediately establish a subquarantine line to the east of that, so that no cattle can get in and reinfest it. That is a precautionary measure, and I think a very good one. It holds that county free from any infection from the outside. In the work we quarantined about 1,500 pastures. That gives you some idea of the magnitude of the work that was done in Oklahoma for this year. In the little talk I made before I gave the conditions there.

SOUTH CAROLINA.

Doctor **Klein**. South Carolina has no law which will enable the State officials to control the movement of cattle within the State. The only law we have enables us to isolate and quarantine animals that are visibly affected with the contagious diseases which are dangerous to life. Under those circumstances we have the right to quarantine a farm and make such disposition of the animals as the welfare of the community may make necessary. However, about a year ago I began in an experimental way, in cooperation with the Bureau of Animal Industry, to test certain methods of eradicating cattle ticks. I have only been able to give such time to this work as I could spare from my other duties. I have succeeded, however, in reducing the infection in Oconee County to about one-half. We have there now 5 areas of infection, and in these we have a total of 11 farms. On 3 of these farms the work of tick eradication was begun this fall. On the other 8 farms work was begun last fall but was not persisted in by the owners of the cattle. Having no authority to quarantine the cattle, I had no means of causing the owners to continue this work.

The greatest obstacle in the way of tick eradication in South Carolina is the small importance of the cattle industry. Very many men there have no cattle except the family milk cow, and very few of them in the State know anything about the Texas fever quarantine line. They do not know that they can not ship their cattle out of the State if they want to. It is only the few men who have herds of thoroughbred dairy cattle who have had orders from States north of South Carolina who have come into contact with the quarantine law and who are therefore interested in tick eradication. These men, however, are rendering great assistance in the endeavors now being made to have a suitable law passed by the legislature at its meeting in January. The members of the South Carolina Live Stock Association and also members of the South Carolina Agricultural and Mechanical Society, which two organizations include most of the influential men of the State, have appointed committees to draft a bill and present it at the next meeting of the legislature, and appear before the committee that may have it in charge, to urge its passage. The chances are greatly in favor of the passage of the bill with an appropriation of a small sum of money to take up the work.

TENNESSEE.

Mr. KITTRELL. Tennessee has a law relating to communicable diseases among domestic animals, but, as Major Pettibone said, it is not thoroughly known among all the people of Tennessee that such a law exists. Although it has been preached from Memphis to Bristol, yet, like the Bible, not all men have heard of it or have felt that they need it in their business. This law is not altogether a perfect law; it has defects, but it has some force in that it has clothed the commissioner of agriculture of Tennessee and the live-stock commissioner with power to make rules and regulations governing the movement and the control of cattle, and it also provides that the boards of health of the various counties of Tennessee shall act under the direction of the live-stock commissioner and the commissioner of agriculture. We have assumed that we have the power to make these rules and regulations. We have assumed that we have the right to accept the propositions made by the Bureau of Animal Industry, and use for our benefit the appropriation lately made by Congress; and we made rules and supplements to our general rules, which I admit were of little value because there was so much of them that people did not read them.

It has been my custom since I have been in office to issue rules and regulations in conformity with those issued by the Bureau of Animal Industry, and I have distributed them to the boards of health. Each member of the board of health in every county in the State received copies, and we inclosed a copy of this law to every county clerk. In visiting these boards of health, to advise with them about the manner of taking up this work, I have asked them about the rules and regulations, and in many cases they have told me that they had not received them. I know that I sent them. They had never been opened, and thus had failed to do what they were intended to do.

I have made it a point during my administration to go over the State with the county institutes held under the auspices of the commissioner of agriculture. Sometimes these were very largely attended. In other cases only 40 or 50 out of a county attended, where there were possibly 2,500 voters. I presented this matter as best I could. Maybe out of that 40, 50, or 75 farmers there were some who came to learn how to grow wheat, others to raise some other crop, and comparatively few to learn anything about the live-stock industry, but I can now begin to see the fruits of that work. While a great many have not felt any benefit from this teaching, some have, and the ideas have been gradually disseminated.

Another thing that has brought about some education among these people has been a few prosecutions and convictions. We have prosecuted three this year, and have convicted all three. Prior to that we had prosecuted three and convicted them all, which was a great lesson to them. When this supplement was issued, and the agents from the Bureau of Animal Industry were commissioned by the commissioner of agriculture and the State live-stock commissioner to enter counties and to enforce rules and regulations for quarantining and disinfection, they were resisted for a while, but some of the counties which most stubbornly resisted are the counties that are most readily cooperating to-day; and I believe that that appropriaton and that method of carrying on this work have been a godsend to Tennessee and will be to all of the Southern States that will avail themselves of it.

We had in Tennessee forty-one counties more or less infected and under absolute or provisional quarantine, but by hard work and by teaching the people we have been able to clean up some of this area; yet most of the results that have been accomplished are not due especially to any efforts of my own or of the inspectors but to the natural location and conditions. We have been able to locate, we think, all of the infection in the State and to fairly control the same. Having done this, we found that some counties were peculiarly located. For instance, a great part of a county would be exposed by an open range, while the other portion was in cultivation or covered with grass and under fence, and that part of the county would be free from ticks. The reason that they have not had relief is simply because the Government, or the Bureau of Animal Industry, did not have full faith and confidence in the people on account of their inactivity in helping to enforce the rules and regulations, and because these herds were exposed to the open range. Now the people who have learned the necessity of cooperating with the Government have appointed county inspectors, and have pledged themselves to cooperate with the Government and with the State and county authorities, and in that way have restored confidence. They have got in touch with one another and are working in perfect harmony, and the result will be, I think, that Tennessee will get about thirteen counties relieved in whole or in part after another year.

The result of this investigation shows—and a recommendation to that effect has already been forwarded to the Bureau—that six counties should be relieved from Federal quarantine for next year. That does not mean that no infection exists in any of them, because the reports show that it does, but the infection is confined, and the conditions are so favorable as to warrant the release from Federal quarantine, as it can be controlled by local quarantine. Outside of these six counties we hope to get partial relief in other counties—that is, get absolute exemption for a great portion of seven other counties, and in two other counties we hope to have the quarantine made provisional or rather modified, so that cattle can be inspected and allowed to move upon a certificate.

We have gone along slowly in this matter. We commenced not knowing how to undertake the work. We were handicapped in two ways. In the first place, we did not thoroughly understand the life and habits of the thing we had to deal with. After learning that, we needed the cooperation of the people, and they did not understand. After giving them a more comprehensive knowledge of the nature of this thing we were handicapped on account of a lack of means to carry on the work.

This State appropriates $2,500 for expenses. This does not include the salary of the live-stock commissioner, but is for the expenses of the inspectors to carry on this work. That is not sufficient for even the force we have, and this force was not sufficient to cover the area. We covered all the area we could, and by another season the same area will need re-covering; but this reenforcement by the Government has enabled us to cover nearly all the area along the southern boundary of the State.

TEXAS.

Doctor **Parker**. The State has had three men actively at work in the investigations most of the time, beginning in the northern part of Texas about the 1st of September and in central Texas about the 15th of September. During a small portion of the time there has been one other inspector on this work. The regular quarantine force of the State for the enforcement of the quarantine line has been doing work of inestimable value in connection with tick eradication, because the enforcement of the quarantine prohibiting cattle from movement is the first essential of our work. If we allow ticky cattle to move there is no use in trying to eradicate the tick; so we can not say that the State of Texas has not helped. Much good has resulted from the work of these three inspectors as a result of their familiarity with the country and their knowledge of the people and of the ranch methods. The Bureau had ten men in the field a part of the time, but the number is now cut down to eight, I believe, and I am afraid it will be cut down some more. We have not had a large force, considering the territory we have had to work in. There are nineteen entire counties and portions of three other counties above the quarantine line in Texas that are more or less infected. One county, I believe, will run as high as 75 or 80 per cent infected; others run less.

In Childress County we have inspected 23 premises, covering 54,260 acres, with 7,875 cattle—selecting those premises which were most likely to be ticky—but we have found no ticks. The inspections were carefully made, and the cattle from many of these ranches were inspected at various times through the summer, and at no time have ticks been found originating in Childress; so we have recommended that Childress be released from quarantine.

Cottle County has 6 premises out of 10 free of infection. There are practically no small farms. Ten big ranches constitute ~~the~~ county, of which 6 are free, covering 25,420 acres, with 4,140 cattle, and 4 are infected, comprising 154,600 acres, with 10,700 cattle. I believe we have gone over all the ranches in Cottle County. They were only slightly infected and on some of the pastures of the ranches no ticks were found. We expect to have Cottle County free from infection by June. The work was done on all of those ranches in Cottle County before the late severe weather, and in most cases the cattle have been transferred to clean pasture, leaving the infected portions vacant over winter. If necessary, extra line riders may be dispensed with. We have reasonable assurance of success in Cottle County, and if ticks are found next spring the ranchmen are posted and will dip as soon as they find them.

Hardeman County has 190 premises with 160,572 acres, and 17,596 cattle free from infection; 39 premises with 80,038 acres, and 6,004 cattle were infected. There are 6 dipping vats, including those which are in or to be put in as rapidly as the carpenters can be had to put them in.

Foard County is heavily infested; 5 premises, with 98,440 acres and 10,035 cattle, were found to be free, and 10 premises, with 68,940 acres and 16,335 cattle, were found infested. There are 7 dipping vats on the 10 ranches. Foard County is about half inspected. The balance will probably show about the same percentage of infection.

Wilbarger County is very generally ticky, but is being rapidly settled by farmers, and we have compelled the small farmers to clean up and show clean premises. We have just struck Wilbarger at the right time. Forty premises, with 28,290 acres and 1,399 cattle, are free from infection; 36 premises infected as far as reported, with 75,500 acres and 9,007 cattle. They have 8 dipping vats in or ordered in.

King County is another which we expect to show free from infection by June 30. There is a very small percentage of infection and it is light, and the pastures are not timbered to amount to anything, nor very rough. Nine premises, with 846,800 acres and 50,400 cattle, are free from infection, and 3 premises, with 19,640 acres and 2,800 cattle, are infected. One dipping vat is in and 2 more are to go in.

In Knox County everything so far as reported is infected. There are very few clean cattle in Knox County; 7 premises are infected, with 124,020 acres, 13,450 cattle, and 4 dipping vats. It will take them two years to clean these up.

The total inspections made in Texas show 315 premises, with 1,349,564 acres and 101,448 cattle, free from infection; 220 premises infected, with 2,582,883 acres, 156,110 cattle, and 69 dipping vats in or ordered in. In addition the live-stock sanitary commission has adopted regulations which will be of great benefit, and the commission will see that these regulations are strictly enforced. As these ranches have to depend on cattle alone for their income, uninfected localities like Sterling and south Mitchell counties have been allowed to drive their cattle over certain trails as southern cattle. That is to stop absolutely January 1.

There are some propositions from the heart of the tick country from people wanting us to take up the work there. These are only isolated cases generally, for of course a ranchman can not afford to raise cattle that are not immune, as long as he depends on the surrounding ticky country for a market for his stockers. He must find a market for tick-free cattle and establish that market before we can persuade him to clean up. I do not think it is advisable to clean up any locality below the line unless we can get the consent of every one in that locality. We must have the sentiment of the people with us. I have thought of asking the legislature to enact a law leaving it to a kind of local option, like the local option on the liquor question, so that whenever a certain number of the people shall apply for an election it shall be held, and if the people are in favor of compulsory measures they shall be put into effect.

VIRGINIA.

Mr. BROWN. The cattle in about two-thirds of the State of Virginia are infected with ticks. The southeastern portion of the State is infected, and it is the work of the board to try to educate the people up to improving conditions. We believe we have got on the right track, and this year we have received most substantial assistance from the Bureau of Animal Industry, enabling us to extend education among the people, and I think that work has borne valuable fruit. As I am simply chairman of the board and not immediately in touch with the work in the field, I shall be very glad to have Doctor Curtice occupy the rest of my time and tell of the actual work that has been done in Virginia.

Doctor CURTICE. In Virginia the work has been done in cooperation with the board of control, the boards of supervisors of the counties affected, and the inspectors—one for each district—appointed by them. These inspectors, about fifty in number, have worked for periods varying from three weeks to a month. Some individuals have continued their work somewhat longer in some of the infected counties. The Bureau force, cooperating, has varied from twelve men in the beginning to six or eight now. In this work the inspectors were sent to a county where they met the local inspectors and talked with them and instructed them, in addition to the instructions that they had received from the State veterinarian in the duties of inspection. We found the best service where we began work together. One objection was that the supervisors, not having very much actual interest, did not want to put up the money to pay for local inspection over a considerable length of time. In some cases they thought they could pay $150; in some $300; but I think in none as much as $500.

A decided advantage in that local inspection service was that good, reliable men were selected and that we were immediately in touch with the cattlemen. We were favorably introduced to the residents of the vicinity, and that counted for a great deal. It removed friction and helped us in our future work. The quarantine of cattle varied from about 15 per cent in some counties to none in one county, or an average of about 6 per cent in ten counties. We believe that 50 per cent of those quarantined are already disinfected, leaving possibly 3 per cent of infected cattle for next year, and 97 per cent to go at large. We have tried to impress the owners of the 97 per cent that they were prevented from going free because they held this small percentage of infected animals; or, in other words, that the 3 per cent of infected cattle were quarantining their 97 per cent. Some of them have come to look at the thing in a new light, and I believe mean business in maintaining quarantine on the infected herds. A certain number of counties have been laid out for work next year.

There has been something said about climatic conditions and natural surroundings favoring certain States. The northern limit of the tick area is bound to be favored by climate, but wherever there is free range there the ticks are, whether it is north or south. Where they have stock laws, where they change their cattle from place to place and have cold weather, there the ticks have been eradicated; and I think this results not wholly from natural causes but partially from the acts of men in handling the cattle.

Doctor KLEIN. I believe the roll call of the States has now been completed. We have one with us who, I think, can say something of interest on this subject who has not yet been heard from, and I, in common with some others, would like to hear from him. I refer to Mr. Dean from Kansas City.

The CHAIRMAN. We will hear from Mr. Dean if he will favor us.

REMARKS BY ALBERT DEAN ON THE WORK IN THE WEST.

Mr. DEAN. Our acquaintance with the tick dates back nearly thirty years, when the great droves of cattle originating in the great State of Texas had to find a market north and were driven over the trails from time to time as settlement moved westward. The prairies of the Indian Territory, as soon as the Indians became subdued sufficiently to allow cattle to stop there to graze, were the great shedding grounds for ticks. Of course we did not understand it at that time. The cattle moved on a little later after they got their fill of grass. They moved into Kansas and eventually on into Nebraska, the Dakotas, and Montana. In the early history of the cattle business, while cattle traveled on foot by trail, Texas fever was never known to exist north of the central part of Kansas; but just as soon as they began to transport the cattle by rail then the fever broke out at the places of landing. It did not make any difference how far north they went, unless they reached some country where they had frost every month in the year.

When the Texas fever quarantine was inaugurated, Oklahoma was still the great grazing ground for the cattle. In our earlier experience we thought it was impossible for fever to hold over from one season to another. Such a condition never existed to our knowledge, and my acquaintance covered twelve years in the cattle business—from 1874 to 1886. I was continuously in the cattle business in what is now the northeastern portion of Oklahoma. During those seasons the grass was not grazed short; the timber was very sparse; the country would be swept every year by prairie fires from one end of it to the other, and instead of a low temperature and a cold winter eradicating the tick, it was in a large measure done by the fire. Just as soon as the country became settled and fenced up and the prairie fires stopped, the ticks apparently secured a hold, and they seem to be capable of great adaptability to different conditions and different climates, especially where they are sheltered. Consequently, when the Texas fever quarantine was applied to Oklahoma—I believe it was in 1897 when the first quarantine line was designated through the Territory of Oklahoma by the Secretary of Agriculture—we found the tick had secured a fairly good hold in that section. The western part of the Territory has a very much higher altitude than the eastern part, and we thought that cut a big figure. We found the tick secured a hold as far west as the 100th meridian and as far north at least as the 36th meridian, north latitude.

Tick eradication is not really a work of recent origin in the Territory of Oklahoma. The rules of the Territorial board and the work of the Bureau of Animal Industry having gotten the cattlemen interested in it, when they understood their position so far as the marketing of their cattle was concerned they set to work to clean up the cattle diseases, and the consequence was that the tick had been entirely eradicated from the counties of Day, Roger Mills, Custer, Washita, and Blaine before ever this recent eradication work set in.

At the point where the national quarantine line crosses the Mississippi River and the boundary line between the States of Arkansas and Missouri we first encounter a low-lying tract of land, commonly known as the sunken lands. It was probably once, according to geologists, an old bed of the Mississippi River. The river has changed from some cause and left a lot of land which is very low. A great deal is covered by water a good portion of the year. This is in northeastern Arkansas. That portion is free from tick infestation as a rule, while the ridges and higher points are infested with ticks.

On the original examination made of that country, about 1895, we found a large number of counties—the two northern tiers of counties of Arkansas—free from infection; but just as soon as a quarantine was established under Secretary of Agriculture Morton and a market made for the cattle the southern cattle from the lower White River and the Arkansas River moved up into those counties and soon infested a large part of them. They are hilly counties, in a large measure, and the ticks found a permanent hold, and a very large number of cattle are now infested with ticks. Beginning at the very northeastern corner, Clay and Randolph are reasonably free from infection now. I know of no permanent infection in those counties. The next county, Sharp, which reaches the boundary line between Arkansas and Missouri at one point, is grossly infested with ticks. The northern tier of counties, clear along the line, is practically free from infection until we reach the very northwestern county, Benton, that adjoins the Indian Territory on the west, and here there is no law—or if they have a law they do not enforce it—that prohibits the free interchange of cattle back and forth. The county is used for grazing—very largely free grazing—so that a farmer with surplus cattle in Arkansas takes his cattle over into Indian Territory to graze, and the Indian Territory cattle come in to find a shipping point.

The Kansas City Southern, or the Port Arthur route, runs down through the western portion of Benton and Washington counties, and the driving in of the cattle from the Indian Territory for shipping purposes has caused infection all around the shipping points. We have made a farm-to-farm canvass of that country and have located all the infected farms or localities. The inspector who made the investigation, being familiar with all those points, can inspect cattle out of that country with a reasonable degree of safety, because he knows whose cattle to let out. Therefore, if the State of Arkansas would pass the necessary laws and create the machinery to put them in force I would favor exempting the northern tier of counties, with the exception of Sharp, from the operation of the quarantine; that is, I would put them under special quarantine and give inspection the year round.

At the northwestern corner of the State of Arkansas the quarantine line turns northerly along the western line of Missouri to the southeastern corner of the State of Kansas. In that portion of the Indian Territory we have no county organizations, but the Department of the Interior, for purposes of recording land allotments, has designated them record districts, which correspond to counties. Now the five northern record districts shut in all of the Cherokee Reservation that would lie north of the north line of the Creek Nation, provided that line was extended east to the Arkansas line. The country lying north of that line comprises the five northern record districts, which are under special quarantine. By amendment No. 6, referred to in Doctor Allen's paper, no tick-infested horses, mules, or cattle are allowed to be placed in that district at all at any time of the year except after being dipped in crude petroleum. This year about 10,000 cattle have been dipped and put in that country. That is tick eradication.

While we acknowledge that there are plenty of ticks in that reservation from year to year, yet when that becomes a part of the State of Oklahoma and gets proper laws we can go after those ticks; and I take it that the object of this work of the Bureau in prohibiting the introduction of any great infection there is to get ready for the time when we get a proper law.

I think it is not out of place right here to state that we have noted no serious effects on account of dipping animals in crude petroleum of the Beaumont variety, with which I suppose most of you are familiar—a low grade of oil, so far as refining purposes are concerned, and containing quite a percentage of sulphur and having an asphalt base. We have a similar grade of oil in the northern part of our oil district in Kansas that has an asphalt base but is lacking in the sulphur, and is a little higher grade by gravity measurement than the Beaumont oil. We have had good results when the Beaumont oil is used under moderate temperature conditions and the animal has a sufficient coat of hair to hold the oil. We have had no trouble in regard to our dipping. Our loss from shipping and dipping and everything else on those reservations to which this dipping order has applied has been less than 1 per cent. We handle the cattle in great numbers.

We now pass on westward to the Osage Indian Reservation, which comprises about a million and a quarter acres of land, that is, including the small Kansas reservation. Last spring a year ago the Department of Agriculture issued regulations prohibiting the introduction of tick-infested cattle into the Osage country until they had been dipped. About 90,000 cattle were dipped under that order. Of course it was opposed by the stock owners largely. They were afraid of it, and they had made their leases with the Secretary of the Interior before this order was out. A good many of them tried to back out and talked about throwing up their leases. The cattle, however, were in good order and the loss through dipping and from all other causes was very small. Some cattle are always hurt owing to the rough usage in handling range cattle; the cattle are wild and jump around. Another difficulty we met with was that the twenty-eight-hour law, so called, did not apply to the Indian Territory—or was not observed, at any rate—so that the cattle were kept on cars for a much longer period than the time limit, and, being exceedingly thirsty, the first thing they did was to take a drink of the dip. We had gutters in the dipping pens, and the oil ran down the gutters and they sipped that oil, and it did not have a good effect on the animals. We think some of the loss was caused by the drinking. We had the nicest, sleekest lot of cattle I had ever seen. Some of them did not fatten as well for the next month. Besides, the shedding of their hair made the animals look as if they were thinner. This year we dipped 125,000 to go into that country, and have this fall put about 8,000 more into the Osage country.

The gentleman from Louisiana said they could not safely use the Beaumont oil in that State; but we can take the Louisiana cattle into Oklahoma and dip them, and we did take about 2,000 from north central Louisiana—that is, from Louisiana west of the river—up to the Ponca or Otoe Reservation, and we dipped them and they did well, and we killed every tick.

Our best inspectors and closest observers estimated that with one dipping under proper conditions, just at the right time of year, when the animal has enough hair to hold the oil, and the oil is of sufficient consistency, not one tick on a thousand animals will stay on.

Of course, we have not succeeded in eradicating all the ticks from the Osage country, for the reason that the Indians do not come under our regulations at all. The Indians have a few cattle, and they have free access to those pastures. While there is nothing in the lease from the Interior Department that gives an Indian any right at all, yet he does as he pleases. He gathers up his cattle at round-up time, and the white man always manages to help him out; and they continually keep those pastures infested with ticks. Of course, that country will come in for allotment presently. Burning would be a good disinfectant for that country, and we have contemplated taking this matter up with our Department and trying to get the consent or backing of the Interior Department to burn that country. We believe it would be a good step toward the eradication of ticks there.

West of the Osage country we reach the Arkansas River along the line, and the rest comes under the Oklahoma regulations. Texas is the only other State that belongs to the Kansas City district, and Doctor Parker will answer for that.

Doctor Allen, the Bureau inspector in charge in Oklahoma, who could not attend this meeting, has sent me a paper, which, if you will allow me time enough, I will read.

WORK AGAINST TEXAS FEVER, WITH SPECIAL REFERENCE TO OKLAHOMA.

By Leslie J. Allen,
Inspector, Bureau of Animal Industry

[Read by Albert Dean.]

The battle with ticks, more for the prevention of their encroachment on free territory than for their eradication, has been waged vigorously—largely by proclamation—ever since the relation between this parasite and the fever has been well understood. I say "by proclamation" for the reason that legislative bodies have never seen fit to enact such measures and provide such appropriations as would give to the live-stock sanitary boards the necessary authority and means for the employment of an adequate force of inspectors even to prevent the gradual invasion of formerly clean territory by infection, to say nothing of carrying into the enemy's country a war of extermination. It can hardly be said, however, that the legislatures have been derelict in their duties to the people, since public opinion, the force that must ever be brought to bear to obtain the enactment of measures intended for a great public benefit or reform, has not given voice to a demand for such legislation. Neither can it be maintained that the public is to an unpardonable degree to blame, for it has never seemed to appreciate properly the relation between the tick and the fever and the fact that to this alone is due the exclusion of their cattle from the northern markets. The owners of the infested herds in a community—suppose, for example, the infested herds are but 10 per cent of the whole—do not seem to recognize the fact that it is due to their harboring of the ticks that the owners of the other 90 per cent are deprived of the privilege of the northern markets. And, strange to say, neither does this 90 per cent seem to be fully alive to the cause operating to that end. While most will agree that it is desirable to be rid of the ticks, still a proper realization of what they are being deprived of would make extremely uncomfortable the position of the man whose disregard for the rights of others is shown by his indifference.

In Oklahoma, too, a Territory the citizenship of which is composed of people from every clime, there is found among the people hailing from those States where it was once believed the quarantine line was established from ocean to ocean for the purpose of eliminating Southern cattle as a factor in the northern markets, a well-marked prejudice against giving credence to the established relations between the tick and the fever. We have another element scarcely less potential, and that is the man from the North who comes to Oklahoma deaf to advice, and fully believing that he, by reason of his fancied superior knowledge of animal husbandry, will be able to demonstrate that ticks have been unjustly blamed for loss from so-called Texas fever. Occasionally one of this type chances to locate his susceptible cattle where the results make a convert. A new convert made in this way is usually very enthusiastic. We also have the pessimist—the man who believes there is no use trying—that ticks originate spontaneously, or as a kind of a budding process from "black-jack" timber.

Undoubtedly this diversity of opinions and apparent want of information is due in a great measure to the lack of a sufficient number of veterinarians and others who are able to explain these matters in such a way as to make them plain to the stockman.

As yet we have had but little trouble with active opposition, and I must say that of what we have had much has been due to a lack of diplomacy or tact on the part of the inspectors. There are comparatively few owners who will actually resist the operation of such measures as are found necessary to accomplish the desired end, providing it can be made plain to them that the measures are necessary and consistent, and that the end to be gained is to their ultimate interest. It is true that there are some who are reluctant to suffer immediate and real inconvenience for the promise of ultimate reward.

In the counties bordering on the quarantine line, where the work was to be begun, it was known that infection existed, but to what degree and its exact location could only be determined by making a systematic canvass. This was done with the twofold object of locating the infection and at the same time preventing as far as possible its further spread by restricting the movement of infested cattle. We therefore organized the force in squads of six, each employee being provided with a mount of two horses. We procured teams, wagons, tents, etc.—a complete camping outfit suitable for our needs. One of the inspectors was designated as manager, to whom all rendered obedience. He thus in a great measure became responsible for the action of all. In this way a nearer approach to uniformity of methods is possible.

One of these squads would start at a given point, say, in the morning, and the managing inspector would designate two inspectors for each of the section lines, the farms on either side of which were to be canvassed. The cook, in charge of the wagon and the extra horses, would be directed to proceed to a certain point previously agreed upon—some 2 to 4 miles ahead, depending upon the character of the country—and make camp and prepare our midday repast. This would be continued in the same direction through the entire county, or to a point agreed upon, where the outfit would double back, taking another 3-mile strip to one side or the other of the one that had just been worked.

The examination of the cattle was usually made in the pasture where found, which was not a difficult matter as a rule, as inspectors who have worked in the West as long as two years or more, if they are made of the right material, become reasonably proficient with the rope, while the class of employees designated as agents in tick eradication, who make up the majority of our force, are men whose recommendations were based, among other things, on their proficiency with the rope. The finding of the cattle in the pasture is the greatest time-consumer, for of the territory canvassed this year probably two-thirds is covered with a dense growth of "black-jack" timber. The ordinary farm in the timbered localities is a 160-acre tract, with some 40 to 60 acres cleared for cotton and corn, the remainder being in the natural growth of stubby timber, which is the pasture for, on an average, 10 head of cattle. Often an inspector will be as long as two hours finding as few as two or three cows on one of these places.

We do not, as a preliminary to making an examination, hunt up the owner to obtain his consent, for such a procedure would occupy too much time, not so much in getting his permission to go on his premises as in explaining the purpose of the crusade and, possibly, in the engagement in a useless controversy on the relation between the tick and the fever. And it might be in order here to pause and remark that not all our employees, even among the professional class, are properly equipped to take a creditable part in such arguments. The discussion of this subject, as far as possible, should be left to the "follow-up" man, who should be selected not only for his special fitness for making convincing explanations of the relation between the tick and the fever, but to advise ably with the owner regarding the best method to employ in his particular case to free his pasture from infection.

When an inspection is made the number of head of cattle, the description of land, and the name and address of the owner are noted. If the cattle are infested with ticks a quarantine notice, the form of which is supplied by the Oklahoma live-stock sanitary commission, is served on the owner.

[Mr. Dean (commenting). This is right in line with a remark I made in regard to the tick eradication not being new to Oklahoma, because that territory has a very efficient sanitary law and they have been pushing the tick back from counties that were only partially affected for five years. It has been successful in eradicating the ticks from five counties entirely. Those counties are Day, Roger Mills, Custer, Washita, and Blaine, and in partially eradicating them from Canadian County, but we never were entirely successful on account of the rough country brakes along the south of the Canadian River, a tract of cedar brakes from which we never have been able to eradicate the ticks by this method of quarantining the premises and trusting entirely to owners to disinfect their cattle.]

This notice apprises the owner of the fact that his cattle are infested with ticks, of which he, in most instances, is fully aware; and he is thereby forbidden to move or allow to be moved any cattle to or from his premises, and is further directed to disinfect them by dipping in or making applications of crude petroleum as often as every twenty days until no more ticks shall appear. This form of notice was not gotten up especially for the tick eradication work, but is a form that was in use before this work commenced. The matter of most importance in this order is that it is a legal notice to the owner that his cattle are infested with ticks and that it forbids the movement to other premises. The order to disinfect will, in most instances, be inoperative unless an inspector makes frequent visits to see that that provision of the order is complied with. The failure to disinfect properly or to employ such measures as are recommended for eradication is not, in many instances, due to open opposition or even to disinclination, but rather to lack of a suitably equipped man to direct his efforts.

A tabulated report of inspections made is forwarded periodically to the Oklahoma City office, where it is recorded. A map is prepared in the office showing the quarter-sections and even smaller tracts—where land is so divided—and the number of cattle thereon. The quarantined cattle are entered on this map in such a manner as to make such herds readily distinguishable from the free. Besides this, each employee while in the field makes his report in duplicate on a blank form provided for that purpose for every individual inspection made. To make this report requires, on an average, an hour or more every day, and this has proven the most distasteful feature of this work. During the season, up to November 1, 10,589 separate inspections had been made by the force engaged in tick location. This covers 114,832 head of cattle. Of these, 1,317 herds, covering a total of 16,927 head of cattle, were found infested. The average number of inspections per day per man was about 12 herds. This average would have been far exceeded had it not been for the unusual amount and frequency of the rainfall. Of the total number of herds inspected a fraction over 12 per cent were found infested.

Regarding actual eradication, the work has not been rewarded with that degree of success that might have been expected. We were handicapped early in the season by being unable to induce dealers to handle oil in sufficient quantities for disinfection purposes. Again, not enough men were assigned to the following-up work. Of course it was only in that area in which the infection was located prior to September 1 that any hopes were entertained of freeing premises of infection. It is impossible at this time to determine with any degree of certainty how many premises have been freed from ticks. We believe, certainly, that on a great many premises quarantined no infection will appear next year. I base this on the fact that, on the reexamination recently made by our inspectors, at least 30 per cent of the herds were found free from ticks. We expect, however, to continue next year to treat all these places as infested.

We are expecting some good results from the quarantining of about 1,350 premises (up to this time), thereby preventing further spread of infection by confining the cattle to one place until they have been freed from ticks. It has been ascertained that in those localities, where not to exceed 10 per cent of the herds are found to be infested now, not longer ago than four to six years the infection was general, and that premises were freed from ticks by greasing the cattle or, if but a few head, by keeping the ticks picked off, but more often this result was obtained by the burning of the pasture (often accidental, it is true) or by permitting it to remain idle a part or a whole of a grazing season, with no thought of destroying the ticks.

[Mr. Dean (commenting). We have learned some good lessons by the burning of pastures accidentally, and it rather backs up my previous statement that the grazing country, which comprises about 3,000,000 acres of land, formerly known as the Cherokee Outlet, was disinfected annually, prior to the time it was occupied as a grazing country, by the tremendous prairie fires that swept it from one end to the other, as at that time the bodies of timber were rather thin, and a big prairie fire would jump an ordinary stream from burning trees or bark. We had some gales and the danger to life of man and beast was great, but such tracts were always disinfected. Last winter a year ago an accidental fire broke out in a portion of the Osage Indian Reservation, which has been used for probably twenty-five years as a grazing ground for Texas cattle. It is the only large tract in either Oklahoma or Indian Territory in which the land has not yet been allotted, and it is leased for grazing purposes in large bodies by the Secretary of the Interior, or under his regulation, and for two years we have been applying the tick eradication rule to that territory. As I said, an accidental fire got out and swept several large pastures, and that fire-swept tract proved to be free from ticks the next year. I think, therefore, that fire is a good disinfectant if we could use it, but unfortunately we can not use it in a fenced country.]

No small per cent of the infection found was of recent origin and traceable to infestation this year by the accidental intermingling of cattle in adjoining pastures or by the introduction of animals—horses or cattle—from more distant infested pastures. The instances of seeming self-disinfection, which may be classed as accidental—that is, the instances of premises becoming freed from ticks with no systematic effort on the part of the owner—assume a place of no small importance when we come to take into consideration the fact that they have more than balanced the numerous instances of infection traceable to the heretofore unrestricted movement of infested cattle, and emphasize the benefits which may be expected from a strict enforcement of the local quarantine. A rigid enforcement of local quarantine, thereby denying to infested cattle the use of the public highways, will develop a factor that will operate in our favor. It is now being brought forcibly to the notice of different owners that they are absolutely deprived of a market, local or interstate, for their cattle until they are free from ticks. This factor is of the greatest importance, of course, to the owners of the larger herds, the local product of whose farms is put into their cattle, thereby making their marketing their sole means of existence. It is believed that this restriction will make it comparatively easy to induce them to make a concerted effort for eradication next spring, at which time in the year disinfection will certainly be more economically effected.

We have had some violations of our quarantine orders; that is, cattle have been moved from quarantined premises without permission. A few of these violations have been wilful and malicious, but it is believed that the great majority of these could have been obviated had we had a sufficient number of inspectors conveniently located to visit these quarantined places as often as every three weeks.

Many of the owners of the larger infested herds have, by reason of the probability of the reappearance of the infection next year and their still being held under quarantine, signified their intention of disposing of the bulk of their herds between now and spring. It is the purpose then to prohibit, under Territorial (or State) authority, the introduction of other cattle to these premises until such premises have been declared free from the suspicion of infestation. The use next year of public pastures quarantined this year will in most instances be prohibited until such time has elapsed as will suffice to destroy the ticks by starvation.

The movement of horses has been mentioned in this paper as a means of spreading the infection. I have known several sanitary officials to speak of this as a danger which is merely possible but too remote for serious consideration, while others prefer to remain silent on this subject. I am not aware that any State or Territory has placed any restriction on the free movement to or within its borders of tick-infested horses. In this the Bureau of Animal Industry seems to have taken the lead in Amendment 6 to Rule 1, Revision 1, under date of October 1, 1906, which regulation, however, affecst only a small part of Oklahoma and Indian Territories. This silence and the apparent reluctance to attempt to restrict the movement of infested horses is due, evidently, more to the want of a satisfactory solution to offer than to a lack of apprehension of the danger. I have in mind a number of cases in the Territory in which all indications point to the ticks being introduced on horses. As recently as last month two cars of tick-infested horses were shipped from a Texas point to Watonga in Blaine County. Okla., a county that for several years has been classed in the safe area. A stockman living there wrote me regarding this incident and mailed me some of the ticks. I was powerless to take any action in the matter except to pass the responsibility up to the secretary of the Oklahoma live-stock sanitary commission, for the reason that Blaine County is not in the area covered by the Bureau amendment referred to. These horses were to have been peddled out over the country, probably going to as many as fifty different places, but I have just learned that Secretary Morris, acting without precedent but by authority of a statute giving his board power to take such action as is necessary to protect the live-stock interests of a community, succeeded in quarantining these horses before they had been sold.

In Greer County, Okla., the horses brought in annually by cotton pickers from Texas are a prolific source of infection. It is unaccountable why some State authorities refuse to admit cattle from an infested pasture, even after dipping and certification, and then admit horses from the same pasture, apparently without a thought of the probable consequences. Then if any infestation appears along the border it is said to be due to the lack of proper patrolling of the quarantine line! So long as there is an infested area and a zone north of it in which ticks may accommodate themselves, just so long will there be cases of infection of apparently spontaneous origin unless there can be some effective measure devised to limit the movement of infested horses.

In the disinfection it is believed that in the majority of instances the periodical application of oil, either by hand or by dipping, will be found the most practicable. However, it is intended to employ such methods as will in each particular case seem to promise the best results. To carry on properly the follow-up work will require one man for every forty or fifty places under quarantine. For the some 1,350 premises now under quarantine it will require for this work alone as many as 35 men, commencing as early as April 1. Many of these men can, if the work under their supervision is effective, be released by August 15 to take up like work in the area that will have been covered by that time by the location force.

The securing of suitable men for eradication work is certainly a matter of the very first importance, and to secure a large number of men that can meet and carry weight with the farmer and stockman, enlist their interest, and enforce necessary measures on the unwilling, will be found exceedingly difficult. It is only men possessing such qualifications, together with energy, alertness, and honesty of purpose, that are of any service. Without such qualifications a man is not only of no service, but his presence is a positive menace to the success of the undertaking.

DISCUSSION.

Mr. MORRIS. Since we began enforcing Indian quarantine it has been the practice in my office to send out a notice every year to every person to burn his pasture, with the alternative that if he does not burn we will put the matter into the hands of the sheriff to look after at his expense. Of course that will still be our plan this year. All those pastures which are quarantined this year and last year receive the same notice this year to thoroughly burn and disinfect.

I also want to emphasize this point in regard to ticky horses in Blaine County. Doctor Allen transmitted correspondence to my office, and after looking carefully over the statutes I figured we could handle that case, and immediately sent inspectors to the neighborhood and got those horses before they were disposed of. They were trailed from the railroad station about 90 miles, but we have got those horses in quarantine all in a bunch, and as far as the Oklahoma board is concerned we shall make a regulation this coming year requiring tick-infested horses to be dipped or disinfected in some other manner before being brought into the Territory; but if we can have a Bureau order behind that it will be better, and we hope we can get it.

Doctor CURTICE. May I ask at what time Mr. Morris asks to have the burning done, or whether he expects the ticks to be hatched out by that time?

Mr. MORRIS. At the time the tick is hatched the grass is too far along. I recommend the burning just as late as possible before the grass starts—that is, so it will not injure the grass too much, say not later than the 15th of April. I do not recommend burning at this time of the year. I think it better to burn later.

KEEPING RECORDS OF INSPECTIONS.

Doctor CURTICE (to Mr. Dean). What system of keeping records of infected cattle have you?

Mr. DEAN. We make up regular reports along with plats. We have the Department furnish us with little township plats on cardboard. Our country is all in sections subdivided into quarter sections. We have little plats for memoranda and have them perforated. The plats are about 3 by 5 inches and show the township and section, with a little space at the bottom for remarks. It was Doctor Allen's idea to keep a record by plats.

Mr. MORRIS. After the inspectors got a little more used to the work of making out reports they did not kick so much about it. It was, of course, a little extra work and wearisome after a hard day in the saddle. They did kick a little, but in the latter part of the season they seemed to catch on.

Doctor CURTICE. Perhaps our experience in keeping records of counties may be of some help. It is not perfect, for I see many things to be corrected. I adopted the card-catalogue system, and when a herd was inspected kept a record of that inspection from the inspector's reports on a 4 by 6 inch card. I kept the records of a county by themselves in alphabetical order. After a county was inspected I took a list of the places on those cards and ordered the proper inspector to fill out a record of his inspection with the date below the first inspection. On some of these cards I have as many as five inspections, some of them by different men, and always a record of what the man intends to do and what he has been doing and the conditions found. By that process I have found that sometimes inspectors when they thought they had records of the scattered herds had missed some. The record enabled me to give them a list that they must look up. It also gave me an idea of the temper of the men on the farms—whether they were actually doing what they said they would do and were making progress. I think that if the volume of work had not been so great I would have kept a record of the uninfected herds; but above all we should keep a careful record from time to time of these infected herds.

FEEDING SULPHUR, DIPPING, ETC.

Mr. RANSOM. The idea has been very prevalent all over the world, wherever there are ticks, that sulphur fed internally will prevent tick infestation. Several years ago the Bureau of Animal Industry took up that question in an experimental way. I do not remember the exact details of the experiments, as they occurred before my time, but the manner in which they were performed was somewhat as follows: Two lots of cattle were kept under exactly the same conditions in every respect, except that one lot received sulphur and the other did not. They were both exposed to tick infestation, and the result was that there was no perceptible difference in the degrees of infestation in the two lots. The people who are working on the tick question in South Africa have performed experiments of similar nature with similar results. It seems to me that the idea is simply a notion. I do not believe there is any basis in fact for the opinion.

There seems to be, however, some relation between the food of cattle, or rather the alimentary processes, I might say, of cattle and the tick. For example, there are certain opinions prevalent among the people that feeding certain fodder to cattle will result in the ticks falling off. We have made observations along that line at the Experiment Station of the Bureau at Bethesda, Md., where cattle which were very heavily infested with ticks were fed at stated times of the day with alfalfa or some other fodder of that sort, and it was found when the feed was thrown into the manger and the cattle began feeding that the ticks would begin to fall from them like raindrops. Before the fodder was put in you would hear a tick fall off occasionally, but as soon as the fodder was put in the ticks began to fall off about like that [tapping on the table]. Of course those ticks were about ready to fall anyway, but we may suppose that they were waiting to fall in a place where the chances of reinfestation of the cattle later would be as good as possible, and that some slight change of unknown character which occurs in the skin of cattle while actively feeding acted as a stimulus to the ticks and caused them to loosen their hold. This peculiarity of the tick may be explained on the theory of natural selection and evolution.

Doctor KEANE. In our California work with the various crude oils in hand dressing and dipping cattle very few of our oils contain sulphur, and some of them have just a slight trace. We find that the oil acts mechanically, and our oils destroy ticks just as well as crude petroleum. In fact, we used fish oil and destroyed ticks. I believe that the action of the oil upon the ticks is purely mechanical, and I think the sulphur has no effect at all. The sulphur being a constituent of the oil may probably prevent a later invasion by ticks, but as far as the destruction of the ticks is concerned I do not think sulphur has any effect at all.

Doctor **Curtice**. I wish to add a little testimony in regard to sulphur. We have been using 20-per-cent oil emulsion upon the ticks, and it kills the ticks as well as the oil does, although we may judge that there is only about 2 per cent of sulphur in the oil. I think that the emulsion when forced into the hair spreads more evenly over the ticks, and that a thin film closes around the tick better possibly than if the oil were obstructed by matty hairs or otherwise; but if one does the work, so does the other. They are both alike in that respect. Of course the work of greasing is detested by the people, and if there were a substitute it would be used much more freely, I think, although more care might be required in handling it. There is a difference of opinion as to how the oil kills the ticks. One view is that there is something poisonous in it which prevents respiration.

Doctor **Parker**. That is not the whole cause. The emulsion is in very small particles which penetrate the spiracles more readily than the oil will do.

Mr. **Ransom**. I believe that is a very good explanation. Of course it is very doubtful if every tick in the application of the oil itself is entirely covered with a film of oil; undoubtedly a great many of them escape being entirely covered, while in the use of the emulsion a large proportion of them would probably get more or less of the oil into their spiracles. Ticks, as a general rule, are very resistant to poisons; that is, they withstand the effects of poisonous substances that kill other parasites very quickly.

Doctor **Parker**. The skin of the tick is not very absorbent?

Mr. **Ransom**. No.

Doctor **Keane**. This calls to my mind a number of dippings which we made with the lime-and-sulphur dip, with approximately 16 pounds of lime and 20 to 25 pounds of sulphur to 100 gallons of water—the regular scab dip. We dipped quite a number of cattle with that mixture, and it was not effective in the destruction of ticks. There is plenty of sulphur and lime, and we found a number of owners of infected ranches were using this dip, because it did not affect the cattle to any extent. The promulgation of theories about the dipping of cattle with sulphur and lime, or any proprietary medicine, always causes a setback in the work; but we found that the use of oil is effective in destroying ticks.

Doctor **Klein**. I would like to ask Mr. Ransom if it has ever been determined in what form the sulphur exists in Beaumont oil.

Mr. **Ransom**. As I am not a chemist, I would not like to answer that. I think we made in our Biochemic Division a good many analyses of oils from different parts of the country, and undoubtedly they have the data in regard to the compound of sulphur which occurs in the oil.

Doctor **Klein**. The reason I asked that question is this: Doctor Nörgaard, who conducted quite an extensive series of experiments testing the various dips for destruction of ticks on cattle, gave it as his opinion after the conclusion of these experiments that sulphur in some oil was the most destructive substance to ticks. I know nothing as to the details upon which he based that opinion. The thing that first drew the attention of the Bureau of Animal Industry, I have been informed, to this crude Beaumont oil, was the fact that it contained a greater percentage of sulphur than could be incorporated mechanically in any oil; that is, there is more sulphur in crude Beaumont oil than you can get in cotton-seed oil if you undertake to mix in some of the flowers of sulphur. The form in which the sulphur exists in the crude Beaumont oil is, I think, a point that will have considerable bearing on the influence of the sulphur upon the tick. If the sulphur is in soluble form, or in a form which will permit it to penetrate the tick, it is certainly then in a condition to do more damage to the tick than if it is in insoluble form or in a condition which will not allow it to penetrate the tick. I have a great opinion of Beaumont oil in the destruction of ticks. I recognize that it is a smeary mess; nevertheless, it does the work. In so far as heat is concerned, I have dipped cattle in Beaumont oil when the temperature was 100 in the shade, and by placing the cattle under a shed they did not suffer any ill effects from the heat. If you dip cattle in Beaumont or any other oil and expose them to the sun the oil will absorb a great amount of heat from the sun, and that is what elevates the temperature of the animals. On the other hand, you will find that crude Beaumont oil in winter time will make cattle shiver. I think that with proper protection from the sun there will be no bad results from the effect of heat on cattle after being dipped in Beaumont oil.

The **Chairman**. I would be glad to hear from Doctor Keane, of California. He said they used an inch or two of oil on water.

Doctor **Keane**. The dipping vats that we used in California are about on the same plan as the Fort Worth vat. There is usually a swim of about 4 feet wide, about 12 feet in depth, 3 feet 6 inches in the clear on top, and a foot and a half on the bottom. It just gives the cattle a chance to go through without turning around. We put about 9 feet of water into that vat and then on the top we measure at first about an inch of oil, and after a few cattle—say 10 or 15—are run through, oil is added at the rate of approximately a gallon to each bullock. We find that a bullock will carry off a gallon of oil. Where the cattle first come into the vat we have a false drop. It descends about 2 feet below the oil and water surface, and the cattle step over and cover the abdomen and parts where the ticks usually invade the cattle, and then after they swim to the middle of the vat we have another false drop. They start to climb up and make another drop, insuring a second immersion in the oil. At first we used hot water, but it broke up the oil into smaller globules. Now we use cold water, and of course the oil remains thick on top, and this has proved very effective. I do not think it is as effective—of course it can not be—as a full oil dip; but if we inspect the cattle and find any more ticks we run them through again.

We usually do the dipping in the evening. Shade is an absolute necessity in the dipping of cattle with oil. We usually dip them in the evening and very slowly, because if you dip fast the oil will mix with the water and it becomes necessary for the oil to rise to the surface again in order to get the mechanical effects of the oil. This dipping has proved very beneficial in our State. I think our oils are just as effective as the Beaumont oils. We have secured results in the destruction of ticks with oil.

We have in a number of counties public dipping vats. We go to the county boards of supervisors, who run the affairs of the county, and when we have a district badly infected with ticks, where there are a number of small ranches, where it would be too expensive to put in dipping vats of their own, and we ask them to put in a vat out of the county funds, and we have been successful. On a great many ranches there are private dipping vats. On smaller places they are not so large, but at the same time they are effective.

I believe that the float dip, in places where you can not use the full oil, and in some places where there are oils that are pretty hard on cattle (some of the heavier grades of oil containing asphalt I believe do the damage to cattle), is a very good substitute for the full oil dip.

The Chairman. I would like to ask Doctor Payne if he has experimented sufficiently with the new oil that he has been trying to throw any light upon the subject, or if he has carried the tests far enough to justify a report.

Doctor Payne. The number of cattle which have been treated or greased with the oil of which you speak—the Kentucky crude oil—has not been sufficient, and results are not sufficiently known, for me to render an official report as to what the results are.

The Chairman. As far as you have gone it has been satisfactory, has it not?

Doctor **Payne**. Yes; in a few individual cases where we have had an opportunity to see the cattle and examine them, we can not see any difference between the Kentucky crude petroleum and Beaumont oil. This Kentucky oil is a grade of oil from which the lighter grades, such as naphtha and kerosene, have been extracted. It seems to have lots of asphalt in it, but so far as we can tell at the present time the results of using it have been very satisfactory where it has been properly applied.

Doctor **Parker**. I do not consider this final as regards the safety and practicability of this dip. This is merely a preliminary report.

Doctor **Mauldin**. How much ought each animal take out of the dip?

Doctor **Parker**. We have no way of estimating it.

Doctor **Curtice**. We have been told that the waste is large where there are large numbers of cattle. We have not discussed yet the cost of disinfection in its various phases, and it comes up to us in the East with great severity when we consider the small numbers of cattle and the fact that they are scattered. In one county in North Carolina we have tried disinfection. The great lesson taught here has been that the work in the West has been under supervision. In the East we have usually permitted the owners of the cattle to do it, and they have failed; but in Yadkin County we have had some supervision. We furnished the oil, the pump, and the outfit to do the work, got the cattle and treated them, turned them loose, and reinspected afterwards. We did everything. To apply one disinfection it took the services of the inspector, with living expenses; we may count that at not less than $7. It took a team to haul the outfit; we can put that down at $3 more. It did take a second inspector, but we could have used a laboring man. Then there were two laboring men at $1 each. The driver, who was only hired to drive, was paid 50 cents for extra labor, making $2.50. The feed for the horses and dinners for the men would add $1.50 at least. I estimate, therefore, that it would cost us about $15 for such a day's work. I asked the inspector how many head he treated daily, and he said from 7 to 15 a day. That seems pretty expensive. With large herds the expense becomes less. We have found that the inspectors need not go once in three weeks, but probably once in four. It appears that before the dipping will clean the herds the expense will be equal, in most cases, to that of abandoning the pasture on September 1, and then putting the cattle on next spring, although reinspection next spring will have to determine the fact. To offset against that expense of dipping we have to consider the continuous rounds and the prospective rounds next year. In the long run those expenditures for continuous inspection add up greatly. I believe that we can make pasture, sow grass for an area, pick up the loose cattle, and give them back to the owner fattened cheaper than we can disinfect them in any other way.

Mr. **Morris**. I think they pay too much up there. We do differently in Oklahoma. The only expense is the expense of the inspector. The owner is notified to have his oil on a certain day and have plenty of help. The inspector goes and supervises it. The man who owns the cattle pays for the oil and for the help. He is told he can do it or pay additional sheriff's expenses, as the latter will buy the oil and will furnish the help. But when a man sees he is up against it he would rather do it himself.

Doctor **Parker**. Have you had a test case of putting the sheriff in charge?

Mr. **Morris**. No, sir; we can also claim that no man has ever tried. A man came in about a month ago to one of the best lawyers in town. There were some $7 expenses against him. The lawyer came to me and said, "How about this?" I showed him the law, and he said, "I will tell him to pay the bill."

Doctor **Curtice**. I should amend my last statement. I would not advise the method for all cattle, but for certain of those that we were convinced we could not hold in quarantine cheaply otherwise—for the tag ends and clean-up.

Mr. **Dean**. I think it is very plain that the same conditions do not prevail in all portions of the country. It has been demonstrated all along the line during this discussion that we could not, with any degree of certainty, agree on a rule that will apply to every condition. We must let every inspector in charge of a district adopt the rules best suited to his district and his people. We think we are following that best suited to our people in the open-range and large-pasture areas.

Doctor **Nighbert**. Doctor Curtice did not mention the expense of making the first inspection without disinfection of cattle or the premises. In the States of Georgia, North Carolina, and Virginia it will cost, at a rough estimate, 20 cents simply to inspect those cattle, let alone the disinfection, for the same reason that the gentleman has mentioned—the cattle are scattered so much. We must keep looking through the brush for the cattle, and you can not visit eight or ten premises a day. If a law were made to force a man, after you find the cattle, to disinfect, that would reduce the expense to the simple cost of inspecting and teaching a man how to disinfect his stock.

DIFFICULTIES OF WORK ALONG STATE BOUNDARIES.

Doctor **Curtice**. There are some questions about interstate work that usually come up that we can discuss to advantage here. I am not getting far outside when I call attention to the line between North Carolina and Tennessee and Georgia. It used to bother us six, seven, or eight years ago, and it still bothers us. I will cite a condition: A little village lies on the State line; the country is peopled by a good many residents who have single cows that they take out to pasture on either side of the town. People come and go; the pastures are infected in one county, and no effort is made to clean them up. An effort has been made to clean up in a county to the south, and yet when they came to the obstacle of cattle coming from the other State they ceased vigorous operations, so that the conditions remain about what they were at the beginning. This is true of the line between North Carolina and Tennessee, and, I believe, between Tennessee and Georgia. The methods of dealing with such cases might be discussed here. Of course we know that it depends on what the people want to do; yet I have an idea that a wire fence along the State line might help some portions, if it were feasible.

The CHAIRMAN. I want that question discussed very much. We are bordering on Alabama and Tennessee and North Carolina. Doctor Payne, Doctor Nighbert, and myself have had a good deal of trouble with this interstate-line proposition. It was the greatest menace we had to our work this year; I think Doctor Payne will bear me out in that. We have Fannin County almost rid of ticks under State provision. When Doctor Payne took charge of the force in our county tick-eradication work he asked me about Fannin County, which adjoins Tennessee. I told him I thought Fannin County was practically free of ticks. He put inspectors to work in the lower part of the county, and found it almost entirely free of ticks; also the middle part. But right up next to Tennessee he found a great deal of infestation, perhaps a mile wide and 2 miles along that line. There was Polk County Tenn., continuing to infest Fannin County, Ga. We did not have an inspector watching the northern part of the line, because we did not want to get into trouble with interstate business. He sent his inspectors up there and they found ticks, and he quarantined nearly all the Georgia cattle in Fannin County; put them in close quarantine. The Tennessee cattle continued to come over and eat at their pleasure, and our people got tired of it. They said: "Why, here, Doctor Payne, you are forcing us to put our cattle in quarantine, and we have not said anything; but you must keep Tennessee cattle off our grass, too." Then, I think, the Doctor appealed to the authorities of Tennessee; anyhow, the result was not satisfactory. The cattle continued to come over, and we objected, and I wrote to the commissioner of agriculture a strong letter, and stated what we were going to do. We said, "We are going to take those cattle up and pen them up." We did not do it, however, because we were a little afraid to. Doctor Payne sent his inspectors up there, and the offending cattle seemed to be mostly milch cows, and they belonged mostly to women, and they did raise some objections, because their living was involved. To make a long story short, after we had about made up our minds to pen up those cattle and put the Georgia law to them, Tennessee stopped work on the 1st of November and withdrew the inspectors.

Doctor ELLENBERGER. There is more done there now than in the early season. The State and county inspectors worked in there not to exceed a month.

The CHAIRMAN. But about the 1st of November you withdrew them?

Doctor ELLENBERGER. We did not work there. The State of Tennessee did about a month's work there.

The CHAIRMAN. Here we have been working for about three or four years to clean up that county, and that infection got in there, and it took one of your special men, Doctor Payne, to come down there and find it out. We have got the law in Georgia, but when Tennessee itself withdrew that local inspection we felt that we could not do anything else; but we did not want to get up any feeling, so Doctor Payne and the State authorities just agreed to withdraw for the present. I would like to know just how to proceed. I think, without any instructions, that Georgia is going to insist next year on quarantining all cattle that come in from Tennessee. Tennessee is commencing above and working down and shoving the ticky cattle upon us. We have to have a starting point and work down, too, so that is the trouble that has confronted us in the State. Where Doctor Payne has not had those conditions to contend with, in the western part of the State—in Stephens, Habersham, and White—I think he will recommend a provisional quarantine. We have had success in that part of the State; but in Fannin County, until Tennessee cleans up, Georgia is hopelessly at the mercy of those ticks above us. Fannin County needs just a little cleaning up in the northern part. We did not want to divide the county, because there is no natural line we could adopt. Besides we find cutting a county in two is very distasteful to the people. You must cut out a whole county unless you have some natural line. We did do that in Rabun County. Fannin County is waiting on Tennessee; there is Gilmer, there is Murray to the west of us, and there is Dade and Walker and Catoosa that we could clean up next year; but unless Tennessee gets busy and cleans up that part of the State that adjoins us, we are at their mercy. I would like to have a solution of that matter, and would like to hear further from Doctor Payne or Doctor Nighbert if I have not stated all the facts in the case.

Doctor ELLENBERGER. You people seem to think we do not have troubles in Tennessee. We have the same conditions you have in North Carolina and Georgia. There is Polk County; there is the Cumberland Mountain country. The people have their cows and yearlings, maybe two or three. They may not own a square foot of ground. It is a pretty hard matter to handle them in many instances. This summer we had no way of taking care of certain classes of territory. The best we could do was to inform those people that they must not have ticky cattle, and it was too late in the season to be positive that that territory would be free of ticks next spring; but if we were able to keep those cattle off the range after September 1, then we would have a fair chance of having the territory free of ticks next spring. The only way to succeed then is to work Polk, Bradley, and James counties of Tennessee at the same time that Fannin and Murray and Whitfield of Georgia are being worked. Earlier in the season it was soon found that the State authorities in Tennessee would not turn over their men to the Bureau to direct the work from the same office. For that reason it was decided that the State people—three deputy inspectors—would take up the work in certain counties, and the Bureau force would take up the work in other counties, and then this fall, along toward the end of the season, we would investigate the counties that the State people thought they had ready for exemption. I think the deputy State inspectors in that county work were employed possibly four weeks, but they really accomplished very little more than greasing the cattle one time.

At the time Doctor Payne called my attention to Polk County it was too late to accomplish disinfection this year, because it was away along in September, the 26th or 28th. It was entirely too late; the cattle had been dropping the ticks on the surrounding country. It takes some time to get people interested and have them make arrangements to take care of the cattle, so that all we could do was to get them interested and get ready for work next year. We find the same trouble in Lincoln County, Tenn.—that is, in the southern portion of the county. There are some little farmers in there; a small portion is fenced. What is under fence is cultivated; cattle run outside, and those people have quite a good many yearlings. If it had not been for the yearlings, I think we would have succeeded in getting the territory practically disinfected this year. It is too hard a matter to get them up and grease them. Our inspectors have a great deal of difficulty in finding these young cattle. When they find them, they have a great deal of difficulty in finding the owner. That, of course, made it difficult for our inspectors to report the names of the owners of the cattle they found. Maybe sometimes several days elapsed before they found out whom the cattle belonged to. If we could have disposed of the young cattle there, and managed to get to each owner and see his cattle and instructed him that he must keep them greased and clear of ticks, as we could not permit them on the range if ticky, I think we would have gotten pretty good results there. It was too late in the season to call attention to the fact that it would be necessary to dispose of the yearlings, but we hope to give everybody to understand that they must dispose of the cattle they can not take care of. But many of the people have no place to care for the cow; they have to depend on the range, and it is a hard proposition.

TROUBLE WITH OX TEAMS.

Doctor **Payne**. The chairman has stated the correct conditions prevailing down there. There is one thing we have to contend with which is not general in any other part of the tick-infested district, and that is the steer-team proposition. The farmers and stock owners in some counties in Georgia rely almost solely upon steer teams for hauling purposes, and that is a very serious thing, and it is a hard thing to combat. To rob a man of his means of transportation deals a serious blow to him, and I do not feel able to state to a person that "you can not move your ox team." It shuts a man off from his market and in a great number of instances it prevents him from making a living. I simply mention it as one of the things to be considered in the mountain counties in north Georgia.

The **Chairman**. Those steer teams are not depended on in the whole State. You must not get that impression. We have a nest of those mountain counties that prefer steers to mules. They raise mules and sell them and use the steers, because it is cheaper, I suppose; some people are able to buy a steer team, but are not able to buy the other teams. After we get through with this tier of counties we are now engaged in, and if we can get three or four counties on the west, we will be out of the steer-team proposition. There are no steer teams after you leave the next tier of counties. We are in the last tier of counties where they use steer teams. You do not find them in middle Georgia and rarely in south Georgia, except for lumbering purposes, or something of that sort. I think we are fighting the hardest battle. I think Doctor Payne has had the hardest battle in the whole territory. After next July, if we are successful now, I think we will have the tick proposition solved in Georgia, if we can continue the work. We will be down into the counties where we have stock laws.

Doctor **Keane**. I can appreciate the difficulties which you must have in a great many of these States. We are very fortunate out West—that is, in the State I represent; but do I understand that the ox teams are infested with ticks? I can not see how it would work any hardship upon the parties who own these infested ox teams to prohibit their moving while infested, because it is no more trouble to clean them than it is to curry horses.

The **Chairman**. I do not think so either, but they differ with us.

Doctor PAYNE. It is not as hard to clean the steer teams as yearlings, but if you allow them to go over the temporary quarantine lines others are going to take the same liberties whether you grant them or not, and it is setting that precedent and allowing steer teams to go over temporary quarantine lines which gives citizens and persons in the district a chance to violate the quarantine restrictions.

The CHAIRMAN. We are managing that fairly well. Doctor Payne with his three very efficient veterinary surgeons is right behind the whole business, and I think it is remarkable that we have done as much as we have in the four short months. The people are falling right in line, and as we go down the interest is increasing, for they know that a calf just below the line is worth $4 and above the line $8; and the best men, the representative men, make those statements at public meetings and say it is true, and the people are becoming educated. I believe the educational feature in this work is the most important part. We get our people together and talk these matters over; we show them the life history of the tick, and prove to them that it is a menace not only to the cattle industry, but to the whole county or State. They are becoming converted, and they are willing and anxious to take hold of this work; but we must, as stated before, heap line upon line and precept upon precept, and continue educating. I do not think we should become discouraged at all, but, on the other hand, I think we should look at it optimistically and never let up, do our work thoroughly, be encouraged, and encourage others.

I am satisfied that Georgia will, with one more year's good work and a little more money, clean up the tick. I believe with Secretary Wilson that, if they will give us time and plenty of money, we will sweep the ticks into the Gulf of Mexico.

REMARKS BY W. H. DUNN.

Mr. W. H. Dunn, of Tennessee, being present on the afternoon of the second day, was called on by the chairman for some remarks.

Mr. DUNN. I used to be in this work, and I am still a friend to the cause. I am fully in accord with this movement and shall be glad to do or say anything whatever that I can to help. The trouble has always been to educate the people as to what ought to be done. They thought we were trying to run something over them, but I am glad to say that the feeling has changed, and I do not think there is a farmer of any prominence or of standing in any community who is not in full accord with the work in Tennessee. It ought to be carried out by the State and by the people to the fullest and strictest letter of the law, and I am heartily in favor—and I said so to-day before the resolutions committee at our meeting of the farmers' institute—of the State of Tennessee making a liberal appropriation, so this work can be carried out. It has been hampered and handicapped by not having sufficient means. I do not know in what way the State of Tennessee could spend its money any better than in helping this cause. I do not know any State that could have better cattle than we can have here. We need State aid.

PLANS, METHODS, AND PROSPECTS FOR TICK ERADICATION IN TEXAS.

By JOSEPH W. PARKER,
Inspector, Bureau of Animal Industry.

The State of Texas contains nearly half of the southern cattle in the United States and about two-thirds of the State is below the quarantine line against splenetic fever. The portion of the quarantine line in Texas is over 500 miles long, has eleven bordering counties on the east, and passes through three counties. Nineteen entire counties and parts of three others above the quarantine line are partly infested with fever ticks.

At the time of the establishment of the live-stock sanitary commission of Texas (1893), the most profound ignorance existed among the generality of cattlemen as to the nature, cause, and method of transmission of splenetic fever. The isolation of the plains, the lack of any previous State oversight of the health of live stock, and the necessities of a frontier country had fostered in the pioneer a contempt for scientific discoveries and a spirit of disregard for the law whenever it best suited his convenience and immediate financial interests. Advice based on scientific knowledge concerning the so-called Texas fever was scoffed at and disregarded with the lofty contempt of "an experienced cowman." So it came about that the work for the control of splenetic fever has been fiercely opposed, the rules and regulations of the State and of the Federal Government disregarded with impunity, and the appropriation for the support of the State live-stock sanitary commission made astonishingly inadequate. The very fact that a purely nonveterinary commission was established and has been so maintained for thirteen years reflects the dogmatic spirit of the Texas cattleman and the Texas legislator.

In the face of these odds the State commission and the Bureau of Animal Industry have for thirteen years maintained an educational campaign, until, aided by nature's own illustrations of the soundness of the "tick theory," and the advancing plowshare, very few people in the counties bordering on the quarantine line or above it have any doubts as to the correctness of the doctrine. With the settling up of the country, too, has come a more civilized regard for the law because it is the law. Smaller margins of profit in all lines have enforced more scientific methods of reducing avoidable losses, so that the prevention of splenetic fever has become a matter of individual concern and study. Public sentiment is aroused in half of the partially infected counties west of the quarantine line, and is awakening everywhere. This, while not showing the rapid progress that might have been made, is substantial progress. At least four counties—Collingsworth, Childress, Kent, and Garza—that were among the partially infected counties eight years ago are now free from infection. Three more—Cottle, King, and Hardeman—we expect to show free from infection, or nearly so, by June 30, 1907, if even a small force can be kept in the field to keep the cattle owners at it until they have done a thorough job. This has been brought about by ranch management directed to that end, dipping vats, and the aid of natural conditions. The four counties first named are on the border of the Territory, where occasional seasons present conditions that the fever tick can not survive—such as severity and length of winters, dry summers, general sparseness of grass and brush, and lack of permanent surface water. A very little systematic effort, with nature's assistance, did the work. The fact that these four counties have been freed of ticks demonstrates the feasibility of the proposition.

As we advance eastward toward a more favorable environment for the tick, the methods employed for its eradication must be more nearly perfect. But nature and the cutting up of the large pastures into farms, with consequent reduction of the number of cattle, their increased value, and the increase of tilled acreage, contribute to help us solve the problems of tick eradication in Texas.

Naturally, it is logical that tick eradication should be first undertaken in the partially infected counties above the quarantine line, where public opinion is prepared and conditions are most favorable for success. This gives nineteen counties and parts of three counties for a field of actual work. Three distinct conditions in tick eradication are presented in this area: (1) Unfenced range; (2) fenced ranches; (3) farms.

Fortunately the unfenced range comprises only a small part of this area, viz, parts of Pecos, Terrell, and Brewster counties, and small scattered areas in other counties. Some half dozen unfenced ranches in Pecos and Terrell counties and one in Brewster County are infected to a slight extent. This involves some 400 to 600 sections of land. I already have assurance from most of the ranchmen concerned that they will put in dipping vats and unite in an effort to eradicate the ticks. The plan favorably regarded is to run drift fences, throw the cattle on certain parts of the range long enough for the other portions to become tick free, then dip the cattle and keep them on the clean range. This will require a year at least. Climatic conditions may be counted on to offset many of the imperfections in the application of this method on an open range. The presence of a great number of sheep on these ranches is a distinct advantage.

In the fenced ranch country I class the counties of Reagan, Irion, Sterling, Upton, Glasscock, Mitchell, Howard, Stonewall, Jones, Fisher, Scurry, Borden, Haskell, King, Knox, Cottle, and Foard. In these counties the ranches yet preponderate over the farms, though Jones, Fisher, and Haskell are being rapidly settled. Hardeman and Wilbarger counties may almost be classed as farming counties, but the preponderance of infection is of course on the larger pastures. The ranches and farms are so intimately associated that the same inspectors do the work and the same methods are used, except that "doping," or hand treatment, is encouraged on the farms. The ranchmen are almost unanimous in the counties of Cottle, Hardeman, Foard, Wilbarger, King, and Knox in their desire to eradicate the ticks and very seldom have we met with opposition that persuasion has failed to overcome. As soon as it became evident that a concert of action could be had the opposition vanished, lumber was ordered for dipping vats, and dipping commenced as rapidly as possible. As far as practicable dipped cattle have been placed on tick-free pastures. In other cases, dipping will be repeated as necessary. Owners of small lots of cattle on farms and in towns have nearly all promised to "dope" their cattle; many have done so, some under supervision. The cattle on farms will be placed on wheat fields or other premises free from ticks, after being themselves freed of ticks. We will show good progress in these counties. Our inspectors in these northern counties of the State are now reinspecting premises previously found infected, reporting what has been accomplished, and advising the owners what further work will be needed.

In Haskell, Stonewall, Jones, Fisher, Howard, Mitchell, and Glasscock counties fewer ranches have dipping vats, and the people have had little experience with any dip or application except hand applications of Beaumont oil obtained from railroad supply, of which they are afraid. They have heretofore been permitted to drive their ticky cattle to certain stations for shipment as southern cattle, or from pasture to pasture, without much restraint. The result was that a great many cattle died last season of splenetic fever—probably an average of 500 in each county. All the shipping stations except Big Springs are infected. Many of the public roads and trails are infected. Public opinion favors tick eradication, but the people are afraid to use Beaumont oil. They do use it, however, when their cattle are dying, but seldom repeat the dipping or doping often enough to eradicate the ticks. Our inspectors are developing considerable influence, but it will be a work of two years or more to educate these people up to the point of doing their work thoroughly.

No work has yet been done in the counties of Fisher, Borden, Irion, Reagan, and Upton, and very little in Stonewall and Pecos counties.

In Sterling and Irion and southern parts of Jones and Fisher counties the inspection, though general, is not permanent. Their normal outlets for shipping are San Angelo, Abilene, Merkle, and Sweetwater, below the quarantine line, and Colorado and Iatan above the line. In these vicinities 50 to 90 per cent of the cattle are infected, but not over 25 per cent are immunes. Heavy losses occur annually. The estimated losses, by counties, for 1906 are—Jones, 500; Fisher, 500; Sterling, 1,500; Irion, 500. Shipping stations at Colorado, Iatan, and Eskota, and trails thereto from the south and some of those from the north, are infected. There have been heavy losses all along the trails, and adjoining pastures are infected. A movement is now on foot by Sterling County cattlemen to ask that Sterling and Mitchell counties be placed below the quarantine line. It is not probable that this movement represents any large portion of the cattle owners in Sterling County, and it certainly represents but very few in Mitchell County. I believe that the temporary damage arising from the enforcement of the law as it applies above the quarantine line will be greatly overweighed by the permanent benefits that will result. This territory is not permanently infested to an extent that the cattle are immunes, and so these counties really belong above the quarantine line.

In Terrell County the Big Canyon Ranch, 8,000 cattle, 160,000 acres, fenced, is the first to begin tick eradication, having a pasture vacated by cattle May 8, 1906, used for sheep since that date. They will put in a dipping vat and dip the cattle to be placed on this pasture, thus vacating another pasture. Other infected fenced ranches of the locality will begin work this winter by the same method.

Investigations are now being made in Upton and Reagan counties. It is probable that these counties are not extensively infected and that good results will be apparent in six months. As their natural outlet is by way of the shipping stations above the quarantine line, they must dip ticky cattle before they can ship them. This insures tick-eradication work in these counties. Owing to the immense area already covered by the force at work in Texas, we have not undertaken work in Throckmorton and Baylor counties, though the effect of the educational campaign is apparent there.

Three State inspectors have assisted in the investigations. The legislature will be asked for an emergency appropriation to enable the live-stock sanitary commission to increase its force of inspectors.

The Bureau force engaged in tick eradication in Texas at present numbers eleven men. Employees are mounted and usually ride two together, following round-ups as far as practicable. In the Colorado vicinity it was found necessary to use hacks to carry bedding and supplies, but this was not advantageous in other localities. In doing the "follow-up" work we expect to dispense entirely with the hacks.

I began work at Quanah, Tex., organizing the force at that time consisting of six men, under Mr. William D. Jorden. They rode two and two and visited ranches when round-ups were in progress. At other times they worked on ranches and had personal interviews with the owners, persuading them to get busy. The results will be shown later. In case we found an infected shipping station, we have had no serious opposition in enforcing the treatment of the cattle with Beaumont oil, so that I believe we will show these shipping stations and small towns in that part of Texas clean. They are clean now to all appearances, having been inspected, reinspected, and treated twice. I have no doubt we will show them clean next season, unless they introduce infection by driving in infected cattle. The same method is being inaugurated on the Texas and Pacific Road, because our shipping stations must be clean to afford an outlet for clean cattle.

Next I organized a force at Colorado, Tex., under Mr. Dan McCunningham. There were four men on that force a part of the time and all the Texas inspectors helping. They have a different kind of country to go through. They can not go on horseback, because they can not depend on the ranches to accommodate even two men offhand. Their horses must be fed, and those ranches do not keep feed for horses. Again, they might find themselves a long distance from any ranch headquarters when meal time or sleeping time comes, so the time lost would be a great deal; therefore we thought it best to rig up a ranch hack, tent, and camping outfit. Two men would go to one pasture and two to another, or one man would go to a round-up, just as circumstances indicated. When cattle are being rounded up by the owner one inspector can do a thorough job, and we take advantage of that fact. When we go into the farming country along the Texas and Pacific, which we intend to work during the bad weather, the men will leave their hack in town. One man has been placed in Stamford, in conjunction with the Texas inspector, and another at Hamlin, near by, to take up the work in Fisher and Stonewall counties. These two can work together if they find it advantageous. We have one other man in Sanderson.

The first essential to successful continuation of the tick-eradication work in Texas is the enforcement of the law against the movement of infected cattle above the quarantine line. For this purpose I would ask that the Federal and State inspectors be distributed so as to afford the most prompt inspection when persons desire to move cattle, and so as to detect violations of the law and secure evidence for prosecutions. The second condition of success is that a sufficient number of employees be kept on tick eradication to keep the work constantly going. If the force should be entirely withdrawn much of the ground that has been gained would be lost within a month or two. The one condition of securing the active cooperation of the ranchmen and farmers is to show them that it pays. We seldom have difficulty in doing that.

The following table shows the work we have done, and, in part, the results:

Condition of tick-eradication work in Texas.

County.	Free from ticks.			Infected.			Number of dipping vats.[a]
	Premises.	Acres.	Cattle.	Premises.	Acres.	Cattle.	
Childress	23	54,260	7,875	....	....	....	....
Cottle	6	25,420	4,140	4	154,600	10,700	4
Hardeman	190	160,572	17,596	39	80,038	6,004	6
Foard	5	98,440	10,035	10	68,940	16,335	7
Wilbarger	40	28,290	1,399	36	75,500	9,007	8
King	9	846,800	50,400	3	19,640	2,800	1
Knox	....	....	....	7	124,020	13,450	4
Jones	14	5,972	424	29	1,066,082	14,452	2
Haskell	3	6,100	809	12	140,020	13,450	1
Stonewall	....	....	....	4	16,350	1,364	0
Howard	7	22,090	1,767	2	33,280	3,480	1
Mitchell	3	19,840	1,450	17	55,424	23,175	20
Glasscock	5	48,780	3,475	4	42,880	2,550	3
Sterling	10	37,040	2,079	46	252,349	17,143	10
Pecos	....	....	....	1	96,000	6,000	....
Terrell	....	....	....	5	325,760	14,200	2
Brewster	....	....	....	1	32,000	2,000	....
Total	315	1,349,564	101,448	220	2,582,883	156,110	69

[a] Includes those already in and those definitely planned.

Mr. Jorden estimates that 100,000 cattle have already been dipped in his territory. Very little dipping has been done in other localities, with the exception of Stamford. Estelline, Acme, Damsite, Chillicothe, and Stamford, which were infected when we began work, have been freed of infection by hand applications of Beaumont oil under the supervision of inspectors. We expect to make uninfected shipping stations of Colorado, Iatan, Hamlin, and other stations, in the same manner, beginning work on them this month.

Investigations are complete in King, Hardeman, Cottle, and Childress counties. The following ranches are believed to be now freed of ticks: White & Swearingen, Cottle County, 100,000 acres, 7,000 cattle; T. B. Yarbrough, Cottle County, 25,600 acres, 3,000 cattle; Fred Fleming, Cottle, Foard, and Hardeman counties, 82,000 acres, 6,500 cattle; other premises—Hardeman County, 10 premises, 19,820 acres, 1,285 cattle; Wilbarger County, 25,000 acres, 5,000 cattle; Mitchell County, 2,020 acres, 235 cattle; total, 254,440 acres and 23,020 cattle, which are clean or may be reasonably expected to be clean. Besides these, the work in progress, on which we have at present no method of knowing results, may be expected to free a great deal more of infection. Lack of thoroughness by the owners will inevitably result in some failures, and unforeseen circumstances will cause others. As the work was only begun September 1, it is, of course, impossible to say that any territory has been actually demonstrated to have been freed of infection so as to justify the removal of quarantine restrictions, nor will this be possible to determine prior to June 30, 1907, or later. These estimates of the results accomplished are based on the conditions found and the work actually done.

REPORT ON ARSENICAL CATTLE DIP FOR TICKS.

By JOSEPH W. PARKER,

Inspector, Bureau of Animal Industry.

Beginning in July, 1906, in cooperation with the live-stock sanitary commission of Texas, which furnished the material for the experiments, I have been testing an arsenical dip proposed by Dr. N. S. Mayo, chief veterinarian of Cuba, for the destruction of cattle ticks. Doctor Mayo's formula and directions for making and using the solution are substantially as follows:

Arsenic trioxid, commercial	8 pounds.
Sodium carbonate, crystallized	24 pounds.
Yellow soap	24 pounds.
Pine tar	1 gallon.
Water sufficient to make	500 gallons.

Dissolve the arsenic in 20 to 30 gallons of water by boiling 30 to 40 minutes. Add water to make 100 gallons. Dissolve the soda in 20 to 30 gallons of water; dissolve the soap (shaved) in the soda solution; pour the tar into this in a fine stream, stirring at the same time. Mix the two solutions. Add enough water to make 500 gallons.

Doctor Mayo has used this only as a hand application, and reported that it killed all ticks without injury to the cattle, even in the extreme heat of Cuba.

On July 31 and August 3, 1906, near Converse, Tex., I sprayed cattle with this preparation and with a modification made by leaving out the soap, and made subsequent observations to determine whether the preparation will kill all ticks, and whether the soap can be dispensed with, as we know that the use of soap in a dip is impracticable in that locality. The cattle—calves, yearlings, cows, steers, and bulls—were in good condition and heavily infested with ticks.

Lot 1, 10 mixed cattle, sprayed July 31. Soap left out of the solution.

Lot 2, 16 mixed cattle, sprayed July 31. Full Mayo solution.

Lot 3, 25 mixed cattle, sprayed August 3. Soap left out of the solution.

The solutions were not well made, as considerable insoluble brown sediment was found in the vessel in which it was prepared. The water is supposed to have contained iron. Light showers fell on lots 1 and 2 about ten to twelve hours after spraying.

Observations.—August 3, most of the ticks on lots 1 and 2 were dead. Ticks in second molt seemed to survive most frequently, though living ticks of all ages were found. Cattle very slightly stiffened. Skin slightly crisped on the surface. August 7, lots 1 and 2 yet showed a few living ticks, mostly in second molt, but a few ovigerous females seemed uninjured by the application. One living seed tick found on the brisket of a cow of lot 2, but it was unattached. Cattle no longer stiff. Injury to skin consisted in slight crisping of the skin, superficially. The spray without the soap seemed fully as efficient as with it, no differences being observable. Lot 3 on August 7 showed results parallel with observations on lots 1 and 2 on the third day. I was prevented from making further observations by press of other work, but considered the observations final as far as the use of the solution with the spraying machine was concerned. On August 15, Mr. L. C. Roosevelt, the operator of the spraying machine, and Mr. Adolf Real, the owner of the cattle, carefully examined some 30 head of these cattle and could find no live ticks.

On September 25, near Quanah, Tex., 3 cows and 2 calves, in good condition, moderately infested with ticks, were dipped in an arsenical solution made according to the following formula:

Arsenic	8 pounds.
Sodium carbonate	24 pounds.
Tar	1 gallon.
Water sufficient to make	500 gallons.

A concentrated solution for 1,000 gallons of dip was prepared by dissolving the arsenic (double the above quantity) in about 60 gallons of water, then adding the soda, and when no sediment was perceptible the fire was drawn and the tar added. One cow was held in the vat a minute.

On observation sixty-eight hours afterwards 50 per cent of the ticks had disappeared; of the remainder, 75 per cent were dead. The hair and skin were perfectly normal. One hundred and seventeen hours after dipping all ticks were dead; calves were slightly burned on the abdomen and brisket; cow that was held one minute in the dip was slightly burned. None of the cattle showed any symptoms of arsenical poisoning. Animals in first-class condition. The observations were made by Mr. William D. Jorden and Dr. Vernon A. Dennis, of the Bureau force.

Other cattle were dipped in November under supervision and found free of ticks within eight days, as follows:

Fred Fleming, Foard County	5,000
J. J. McAdams, Foard County	2,000
Early Brothers Foard County	1,000
Millard Young, Foard County	35
J. J. McAdams, Cottle County	3,000
M. M. Hankins, Hardeman County	500
W. R. Wheat, Hardeman County	100
Total	11,635

Mr. Givens Lane, a Bureau agent, supervised the dipping of these cattle and made the subsequent examinations. In his report on the dipping of the McAdams cattle he says: "I did not examine a single cow but what the ticks were either dead or in a dying condition, say, after three days." He found no living ticks after eight days, and no injury to the cattle.

In connection with the dipping of the Wheat cattle, 2 cows were dipped in a double-strength solution without injury to them. Mr. M. M. Hankins, live-stock sanitary commissioner, Quanah, Tex., says of these:

> After twenty-two hours, upon examination, the cows were not injured in the least. The male ticks were dead. The large female ticks were very much swollen and had a bluish appearance. The small ticks were practically all dead. The only casualties attending the dipping were two steers dead, and in my opinion they died from drinking the solution which had been carried out by the cattle into the pen, where the cattle stayed over night, and the cattle during the night evidently drank from the puddle of the solution.

In making the dip for these dippings a zinc pan holding about 120 gallons was used, the arsenic and soda being placed in it together and boiled until dissolved, the tar added, and the mixture diluted to the strength desired. No dripping floor was used. At one dipping the vat contained ice and no bad result was noted.

Beginning about November 15, Swenson & Co., Stamford, Tex., dipped 886 ticky cattle and placed them on feed. Doctor Dennis had an excellent opportunity to observe the results. He reported November 26 that on examination that day he was unable to find a single living tick. I helped him examine these cattle November 20, finding a very few living ovigerous female ticks and they plainly showed that they were in a dying condition. Inclement weather prevented his making another satisfactory examination until November 26. It apparently required longer for the ticks to be killed at Stamford than at Quanah, the difference being probably due to the water used or to the manner of preparing the dip. At Stamford the arsenic and soda were dissolved separately, being boiled by steam, then mixed, and not subsequently boiled. Or it may be that the capacity of the vat at Stamford was not correctly estimated and the dip was consequently too weak.

The dangers to be apprehended from the use of the arsenical dip are those resulting from careless preparation of the dip and from improper disposition of the residue in the vat when it becomes necessary to clean the vat. As the dip can be made in concentrated form, 50 gallons to make 500 gallons, the first of these dangers can be overcome. The second danger can be guarded against only by supervision and instructing the users as to a safe method of disposing of the residue.

RESOLUTIONS.

The committee on resolutions offered the following, which were unanimously adopted:

Representing the States of Alabama, Arkansas, California, Florida, Georgia, Kentucky, Louisiana, Missouri, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia, which are more or less infested with the cattle fever tick, and which are suffering great annual losses in consequence thereof, having met in convention to discuss the work accomplished in tick eradication by the United States Department of Agriculture, in cooperation with the proper officials of the several States above named; and

Whereas the most conservative estimates of the direct annual loss resulting from the presence of the said fever ticks amounts to the sum of $40,000,000 annually; and

Whereas the expense of the entire eradication of the said fever tick in the United States will be far less than the direct loss resulting from the same in any one year and will result in a permanent saving of this great sum of money to the cattle interests of the United States; and

Whereas we realize the necessity of a progressive campaign for the eradication of the cattle tick; and

Whereas Congress at its last session appropriated the sum of $82,500 for the prosecution of the work during the current fiscal year; and

Whereas the Federal Government is asking the cooperation of the various States whose interests are so vitally affected; and

Whereas very beneficial results have been obtained by the expenditure of the sum appropriated by Congress, and the entire eradication of the cattle fever tick is only a matter of continued intelligent effort and an expenditure of a sum of money, very small compared to the great annual loss resulting from these ticks: Therefore, be it

Resolved, That we heartily commend the action of the last Congress in making the said appropriation of $82,500.

Resolved, That we recommend to Congress the appropriation of $250,000 asked for by the Secretary of Agriculture, to be used by the Bureau of Animal Industry of said Department in continuing this work of tick eradication in cooperation with the proper officials of the States concerned during the coming fiscal year, and we further recommend that of this amount $50,000 be made available for immediate use, so that this important work will suffer no interruption.

Resolved, That we urge upon the members of the legislatures of these States the importance of having the necessary laws to empower their State officials to enter into said cooperation, and the necessity of a sufficient appropriation of money to carry on their part of the work.

On motion of Doctor Cary, a unanimous vote of thanks was tendered to Hon. James Wilson, Secretary of Agriculture, and Dr. A. D. Melvin, Chief of the Bureau of Animal Industry, for their earnest work along the line of tick eradication and for their efforts to secure the appropriation of funds for this object.

Doctor Keane offered a resolution, which was unanimously adopted, expressing appreciation of the work of Dr. R. P. Steddom, chief of the Inspection Division of the Bureau of Animal Industry, and his force of inspectors, and pledging support to the work.

On motion of Professor Willoughby, a vote of thanks was tendered to Major Wells, custodian of the building in which the meeting was held, and to other authorities, for courtesies extended in the use of the court room and offices.

REMARKS IN CONCLUSION.

The CHAIRMAN. I am optimistic as to tick eradication in Georgia. I know that we have had some ups and downs, but all of you have had the same. I am satisfied that if Doctor Payne is sent back to Georgia with an increased appropriation, we are going to rid twelve or fifteen counties of the tick. You have encouraged me, and I shall go back home and tell our people that this is the greatest work that Georgia could engage in, and I believe every word of it. We want more cattle. We must have better lands and fewer ticks. We shall never be a prosperous State—none of our Southern States will prosper—until we grow more cattle. We have grown cotton until we have nearly exhausted our land. The West must look to the South for some cattle. We must get ready for this. There is no long grass growing free in the West. You can not start with a bunch of cattle in the Dakotas and drive them to Chicago, taking six months in the driving, and expect them to be fat when they get there. Already Armour and Swift and the other packing houses are looking for more cattle. We have the climate, but we have too much cotton and not enough cattle. If the Government will help us to rid our country of this cattle infection, we will show you the grandest country that the sun ever shone on.

Let us put our shoulders to the wheel and push, and back up the great work that the National Government has done for us. We must do our part. They have done their part nobly, and I feel ashamed that my State has not done more in this tick eradication work than it has in the past, but if we meet twelve months hence you will find that Georgia has not made a small appropriation.

Doctor DAWSON. I believe it would be well for this assembly to appoint a committee to confer with the Agricultural Committee in the coming Congress.

Professor WILLOUGHBY. Further extending the remarks of the gentleman as to the National Government helping the States that help themselves, most of this should be considered as a national problem in the same way that foot-and-mouth disease was considered a national menace to our country, although it was localized in New England, and the same way that scab and other diseases in the West, which might have spread all over our country, were considered a national problem and were taken up by the National Government. So, in the same way it seems to me that the Northern States—all States of our Union—should be perfectly willing to engage in this national problem of tick eradication. It not only affects the Southern States directly, but it affects every State in the Union.

At the meeting of the Southern commissioners of agriculture last fall at Richmond, Va., a committee of five members was appointed which did excellent work in presenting the claims of our people to the

Southern Congressmen and Senators. I have not heard that the members at Jacksonville have continued that committee, and it seems to me that if we should adjourn without appointing such a committee there would be a division in that respect.

The **Chairman**. I think you are quite right. I know the question was up at Jacksonville before the commissioners of agriculture, and they very heartily indorsed the work and asked for an increased appropriation. They urged their Congressmen and Senators to ask for this increased appropriation. I am not advised as to whether they appointed a committee. I think it is very necessary to appoint a committee to act in conjunction with that committee. Or if they did not appoint a committee we should act alone. I would like to hear some discussion on that line or some suggestions. I think Mr. Willoughby's idea is right.

Doctor **Keane**. I believe we should all resolve ourselves into committees of one and use our influence with our Representatives in Congress and try to have them do the same with northern members in Congress. It will do more good than any committee.

The **Chairman**. That is all good work. I, with Doctor Redding, represented Georgia before that Congressional committee last February, and we had representatives from a great number of States. They heard us, and I think it was through our work with that committee that we got that appropriation. As Doctor Keane says, let us all go back home and appoint ourselves committees of one to see our Congressmen. I know last year I wrote to every Congressman we had, and I had a favorable reply from every one except one, and that one did as much to get the appropriation as any of the others. I think if every man will do that it will be effective.

On motion of Doctor Klein a unanimous vote of thanks was extended to Mr. Wright for the able manner in which he presided over the meeting.

The meeting adjourned.

INDEX.

Page.

- Alabama—
  - Report, 47
  - Résumé of work done, 18
- Allen, Leslie J. Paper on "Work against Texas fever with special reference to Oklahoma", 66
- Arkansas—
  - Remarks by Albert Dean, 63
  - Report, 47
  - Résumé of work done, 16
- Appropriations—
  - Congressional, urged, 20, 92
  - Georgia, 8, 93
  - Louisiana, 53
  - Need of, 82
  - State, urged, 8, 20, 82, 92
  - Tennessee, 58
- Arsenical dip, report on, 89
- Beaumont crude petroleum. (*See* Oil.)
- Brown, John Thompson—
  - Remarks, 41
  - Report regarding Virginia, 60
- Bureau of Animal Industry—
  - Representatives present, 9
  - Work of tick eradication from standpoint of. Remarks by A. D. Melvin, 10
- Burning, disinfection by, 62, 68
- California—
  - Law, existing, 29
  - Methods of work, 26
  - Résumé of work done, 15
- Cary, C. A.—
  - Education and publicity as an aid to tick eradication, 40
  - Remarks, 21
  - Report regarding Alabama, 47
- Chairman. (*See* Wright, R. F.)
- Committees, appointment of, 9
- Congress—
  - Commended for making appropriation, 92
  - Urged to make further appropriation, 92, 94
- Cooperation between States and Federal Government, 35
- Crop rotation as an agency for tick eradication, 20
- Curtice, Cooper—
  - Cost of inspection and dipping, 76
  - Difficulties of work along State boundaries, 77
  - Methods of keeping records, 71
  - Remarks, 24, 34, 46
  - Report regarding North Carolina, 53
  - Report regarding Virginia, 60
  - Sulphur in oil, 73
- Dawson, C. F.—
  - Remarks, 21
  - Report regarding Florida, 48
- Dean, Albert—
  - Keeping records, 71
  - Remarks, 77
  - The work in the West, 61
- Delegates, list of, 8

Page.

- Dip—
  - Arsenical, report on, 89
  - Lime and sulphur, 73
  - Oil, 64, 74
  - Oil on water, 27, 74
- Dipping cattle to destroy ticks—
  - Cost, 76
  - Experience, methods, and results, 26, 64, 74–76
  - Good results from oil dipping, 64, 73
  - Vats in California, 26, 74
  - Vats in Texas, 59, 85
- Disinfection of cattle and premises. Remarks by H. A. Morgan, 20
- Dunn, W. H. Remarks regarding work in Tennessee, 82
- Education and publicity as an aid to tick eradication. Remarks by C. A. Cary, 40
- Ellenberger, W. P.—
  - Difficulties of work in Tennessee, 79
  - Elected secretary, 7
  - Report regarding Kentucky, 51
- Eisenman, F. T. Report regarding Kentucky, 50
- Florida—
  - Conditions in, 21, 22
  - Report, 48
- Georgia—
  - Difficulties of work along Tennessee boundary, 78
  - Law, existing, 29
  - Report, 49
  - Résumé of work done, 18
  - Trouble with ox teams, 81
  - Work of tick eradication, 7, 8
- Government, Federal, cooperation with States, 35
- Hand dressing with oil, method of, 23, 24, 25
- Horses, ticks on, 64, 69
- Indian Territory. Remarks by Albert Dean, 63
- Inspection—
  - Cost, 76
  - Methods of keeping records, 68, 71
  - Statement by States, 14
- Inspectors—
  - Need of knowledge of farming conditions, so as to give advice, 40
  - Short course in agriculture, 41, 45
- Interstate Live Stock Association, announcement, 47
- Keane, Charles—
  - Dipping in oil on water, 74
  - Lime-and-sulphur dip, 73
  - Remarks, 9, 26
  - Sulphur and oil, 72
- Kentucky—
  - Law, existing, 29
  - Report, 50
  - Résumé of work done, 17
- Kittrell, R. H.—
  - Remarks, 22
  - Report regarding Tennessee, 56
- Klein, L. A.—
  - Remarks, 22, 34
  - Report regarding South Carolina, 55
  - Sulphur in oil, 74
- Law, proposed uniform, for enactment by States, 30
- Laws—
  - State and Territorial, existing, summary, 13, 29
  - States urged to enact, 92
- Legal side of the tick-eradication problem. Address by George P. McCabe, 28
- Lenton, W. Report regarding Arkansas, 47
- Lime-and-sulphur dip, 73
- Louisiana—
  - Conditions in, 16
  - Report, 52

Page.

- Luckey, D. F.—
  - Cooperation between the States and the Federal Government ... 35
  - Remarks ... 23, 47
- McCabe, George P. Address on "The legal side of the tick-eradication problem" ... 28
- Melvin, A. D.—
  - Remarks ... 33
  - The work of tick eradication from the standpoint of the Bureau of Animal Industry ... 10
- Missouri—
  - Law, existing ... 30
  - Résumé of work done ... 16
- Morgan, H. A.—
  - Disinfection of cattle and premises ... 20
  - Remarks ... 21, 27
- Morris, Thomas—
  - Enforcement of quarantine regulations ... 37
  - Remarks ... 25, 70, 77
  - Report regarding Oklahoma ... 54
- Newell, Wilmon—
  - Remarks ... 25, 27
  - Report regarding Louisiana ... 52
- Nighbert, E. M. Remarks ... 25, 77
- North Carolina—
  - Law, existing ... 29
  - Report ... 53
  - Résumé of work done ... 18
- Oil—
  - Beaumont crude petroleum ... 22, 23, 24, 64, 74
  - Cost ... 23, 27
  - Dipping ... 64, 74-76
  - Disadvantages ... 23, 27
  - Efficacy in destroying ticks ... 22, 23, 24, 64, 74
  - Hand application ... 22, 23, 24, 25
  - Kentucky crude petroleum ... 76
  - Sulphur in ... 72-74
  - West Virginia black ... 23
- Oklahoma—
  - Enforcement of regulations ... 37
  - Law, existing ... 29
  - Paper by Leslie J. Allen ... 66
  - Remarks by Albert Dean ... 62
  - Report ... 54
  - Résumé of work done ... 15
- Order of business ... 9
- Parker, Joseph W.—
  - Paper on "Plans, methods, and prospects for tick eradication in Texas" ... 83
  - Remarks ... 24, 44
  - Report on arsenical dip ... 89
  - Report regarding Texas ... 58
- Payne, A. J.—
  - Kentucky crude petroleum ... 75
  - Report regarding Georgia ... 49
  - Trouble with ox teams ... 81
- Persons present ... 8
- Pettibone, A. H. Remarks ... 43
- Publicity and education ... 40
- Quarantine—
  - Regulations, enforcement, remarks by Thomas Morris ... 37
  - Regulations, publication and notice ... 34
  - Special individual, inside of quarantined area ... 38
- Ransom, B. H. Remarks on effect of sulphur ... 72
- Regulations. (*See* Quarantine.)
- Reports from States ... 47
- Resolutions—
  - Adopted ... 92
  - Appointment of committee on ... 9

Page.
Rotation of crops 20, 21
South Carolina, report 55
State boundaries, difficulties of work along 77
States—
  Cooperation with Federal Government 35
  Reports from 47
  Represented 8
  Urged to enact laws and make appropriations 92
Steddom, R. P. Report of cooperative work 12
Sulphur, effect of, in feed or dip 72–74
Teams, ox, trouble with 81
Tennessee—
  Difficulties of work in 79
  Law, existing 29
  Remarks by W. H. Dunn 82
  Report 56
  Résumé of work done 17
Texas—
  Law, existing 30
  Plans, methods, and prospects for tick eradication, paper by Joseph W. Parker 83
  Report 58
  Résumé of work done 15
Ticks, life of 27
Virginia—
  Law, existing 29
  Methods and conditions 41
  Report 60
  Résumé of work done 19
Willoughby, C. L. Remarks 42
Wright, R. F.—
  Difficulties of work along State boundaries 78
  Elected chairman 7
  Georgia, conditions and outlook 82
  Remarks 7, 93
  Trouble with ox teams 81
  Uniform laws 36

O

636 U 58

Issued May 11, 1907.

U. S. DEPARTMENT OF AGRICULTURE,
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A. D. MELVIN, CHIEF OF BUREAU.

INVESTIGATIONS IN THE MANUFACTURE AND CURING OF CHEESE.

VII.—DIRECTIONS FOR MAKING THE CAMEMBERT TYPE OF CHEESE.

BY

THEODORE W. ISSAJEFF,
Expert Cheesemaker, Dairy Division.

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Page.
Rotation of crops........ 20, 21
South Carolina, report........ 55
State boundaries, difficulties of work along........ 77
States—
  Cooperation with Federal Government........ 35
  Reports from........ 47
  Represented........ 8
  Urged to enact laws and make appropriations........ 92
Steddom, R. P. Report of cooperative work........ 12
Sulphur, effect of, in feed or dip........ 72–74
Teams, ox, trouble with........ 81
Tennessee—
  Difficulties of work in........ 79
  Law, existing........ 29
  Remarks by W. H. Dunn........ 82
  Report........ 56
  Résumé of work done........ 17
Texas—
  Law, existing........ 30
  Plans, methods, and prospects for tick eradication, paper by Joseph W. Parker........ 83
  Report........ 58
  Résumé of work done........ 15
Ticks, life of........ 27
Virginia—
  Law, existing........ 29
  Methods and conditions........ 41
  Report........ 60
  Résumé of work done........ 19
Willoughby, C. L. Remarks........ 42
Wright, R. F.—
  Difficulties of work along State boundaries........ 78
  Elected chairman........ 7
  Georgia, conditions and outlook........ 82
  Remarks........ 7, 93
  Trouble with ox teams........ 81
  Uniform laws........ 36

O

636 Ua 57

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., March 22, 1907.

Sir: I have the honor to transmit herewith, and to recommend for publication as Bulletin No. 98 of this Bureau, a paper by Theodore W. Issajeff, giving directions for making the Camembert type of cheese. This paper is based upon results of the investigations in the manufacture of European varieties of soft cheese which have been in progress for some time at the Storrs (Conn.) Agricultural Experiment Station by cooperation between that station and the Dairy Division of this Bureau, the author being an expert cheese maker on the cooperative staff.

Readers who may desire fuller information as to the nature and character of Camembert and other soft cheeses, the molds used in their ripening, the methods followed in their manufacture in Europe, etc., are referred to the following publications of the Bureau: Bulletin No. 71, "The Camembert Type of Soft Cheese in the United States;" Bulletin No. 82, "Fungi in Cheese Ripening: Camembert and Roquefort," and an article on "Soft-Cheese Studies in Europe" in the Twenty-second Annual Report (for 1905).

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

INTRODUCTION.

For the past three years cooperative experiments have been conducted between the Storrs (Conn.) Agricultural Experiment Station and the Dairy Division of the Bureau of Animal Industry, United States Department of Agriculture, for the purpose of determining the methods of making and ripening cheese of the Camembert type. This is one of the varieties of European soft cheese imported in considerable quantities and considered by many a great delicacy. There is a growing demand for cheese of this type in the United States, and there is no reason why the industry of making this cheese should not be developed in this country. The directions given in this bulletin are not mere compilations, but are founded upon research work covering a period of more than three years. The Storrs Station is now prepared to assist factories and individuals in undertaking the manufacture of this type of cheese.

L. A. Clinton,
Director Storrs Agricultural Experiment Station.

CONTENTS.

Page

The cheese-making plant 7

  Equipment of the making room 7
  Equipment of ripening rooms 8
  Construction and condition of the rooms 9
  Protection against insects 11

The making of the cheese 11

  The milk 11
  Ripening the milk 11
  The starter 12
  Adding the rennet 12
  Cutting the curd 12
  Dipping the curd into the forms 13
  Inoculation and turning 14
  Salting 15
  Making cheese from uncut curd 16
  The use of the low forms 16

Ripening the cheese 17

  Factory methods 18

Various defects of cheese 18

Estimated equipment for a factory 20

ILLUSTRATIONS.

Page.

FIG. 1. Titration apparatus for determining acidity of milk 7
2. Curd knife and dipper 8
3. Large and small hoops, or forms 8
4. Draining board 9
5. Draining mat 9
6. Cane bottom for ripening cheese 10
7. Cheese boards, mats, and forms as arranged for draining cheese 14
8. Method of turning cheese 15
9. Salting the cheese 15
10. Gassy curd 19

DIRECTIONS FOR MAKING THE CAMEMBERT TYPE OF CHEESE.

THE CHEESE-MAKING PLANT.

The first problem to be considered is the construction of a suitable plant in which the cheese is to be made and ripened. The description which is here given is not of the plant in which our experiments have been carried out, but is rather of one which is designed to meet certain requirements discussed later, and which experience has taught us would be most satisfactory.

The plant suggested consists of three rooms, the first of which is used for the making of the cheese, the second for growing the molds and for the first stage of ripening, and the third for the subsequent and final ripening. The size of these rooms depends chiefly upon the quantity of milk which is to be handled.

EQUIPMENT OF THE MAKING ROOM.

Vats.—For the making of Camembert cheese an ordinary flat-bottomed cheese vat is just as satisfactory as the basins used in France.

Apparatus for determining ripeness.—A Marshall rennet test is useful in testing the ripeness of the milk. A more convenient and accurate apparatus, however, is one for determining the percentage of acidity, and consists of a burette connected by a siphon to a large bottle of a one-tenth normal solution of caustic soda (N/10 NaOH). (Fig. 1.)

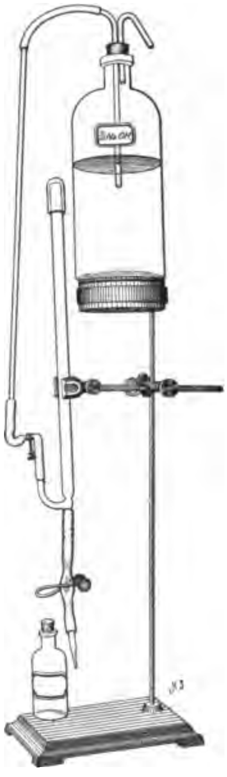

Fig. 1.—Titration apparatus for determining acidity of milk.

Curd knife and dipper.—A curd knife of the ordinary type must be provided in case the curd is to be cut, and also a dipper similar in shape to a soup ladle. (Fig. 2.)

Draining table.—The draining table, one end of which is a little higher than the other, is placed near the vat. The top of this table slopes somewhat from both sides toward the center. It is best to have the table on wheels, so that it will be movable.

Hoops, or forms.—The hoops in which the cheeses are made are cylindrical in shape and open at both ends. They are made of galvanized iron, are 5 inches in height and 4 inches in diameter, and are provided with three rows of holes about 1 inch apart. The size of the holes is about one-eighth of an inch, and there are thirteen holes in a row. A second set of hoops, 2 inches in height, with one row of holes around the center, is made with a slightly larger diameter (one-eighth of an inch larger is sufficient), so that they will slide freely over the others. (Fig. 3.)

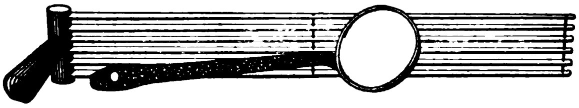

FIG. 2.—Curd knife and dipper.

Boards.—The draining boards are made of whitewood and have parallel grooves on both sides to enable the whey to run off readily. These grooves are about one-sixteenth of an inch wide and of the same depth, and are about one-eighth of an inch apart. The boards are about 14 by 15 inches in size, or large enough to hold nine cheeses of common size. (Fig. 4.)

Mats.—Square mats of the same size are needed to cover these boards. They are preferably made of fine bamboo strips, closely fastened together with strings. They resemble somewhat the bamboo strip curtains. (Fig. 5.)

Cane bottoms.—Cane bottoms are often used. They are of the same size as the draining boards and are used as supports for the cheese during the ripening process. (Fig. 6.)

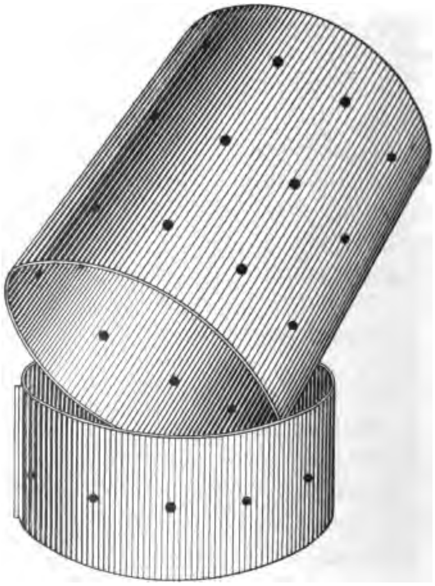

FIG. 3.—Large and small hoops, or forms.

EQUIPMENT OF RIPENING ROOMS.

The equipment necessary for the ripening rooms consists of shelves on which the cheeses rest and means for controlling at all times the temperature and moisture of the rooms. The shelves are made of hardwood and are about 5 inches apart, so as to allow the boards and cheeses to slide in and out freely. They are built from floor to ceiling in order to economize space. Steam and brine pipes will best furnish the means of controlling temperature and moisture.

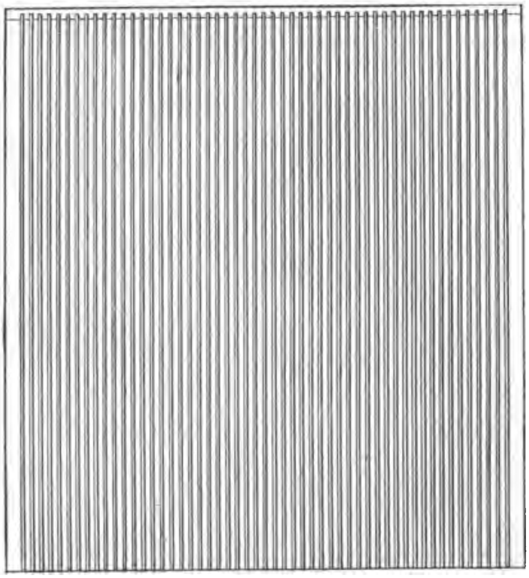

FIG. 4.—Draining board.

CONSTRUCTION AND CONDITION OF THE ROOMS.

Making room.—One of the first requirements is that of absolute cleanliness. The floor should be of cement or some other water-tight material, and should slope toward a drain-pipe, so that it can be readily flushed with water. The walls can be made of wood or brick, preferably the latter, and should be covered with whitewash or enamel paint. This coat of whitewash or paint should be renewed from time to time after cleaning off any dirt that may accumulate, and also for the purpose of disinfecting the room if this should be needed.

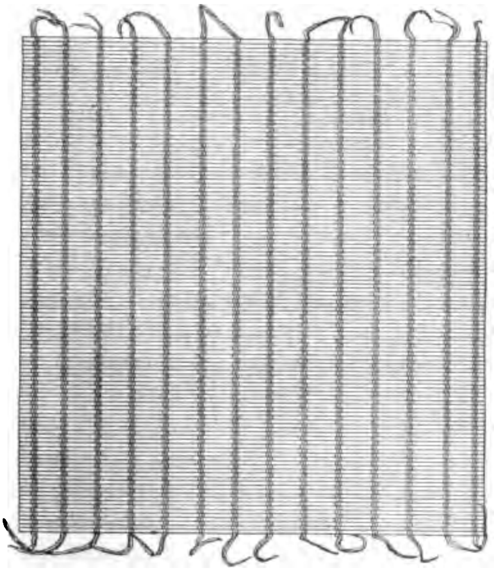

FIG. 5.—Draining mat.

The room must be frequently ventilated, no matter what the temperature of the outside air may be, and yet it is to be maintained at a constant temperature. For this purpose steam should be provided, as stoves or other heating arrangements do not warm the room as quickly or satisfactorily.

An ordinary dairy sink, with water and steam taps, is necessary. The steam pipe should connect with the water pipe by a tee, so that the water can be heated to any desired temperature.

As the tools can not be properly cleaned with hot water alone, it is advisable to provide a steam chest or sterilizer of some sort where they can be left in contact with live steam. A strong wooden box, lined with galvanized iron and having a valve at the bottom as an outlet for condensed water, has been found to be very satisfactory. It is provided with a strong cover, which can be fastened to the box with clamps. The whole arrangement should be made so as to stand a slight pressure. This box is especially useful for sterilizing the boards and cane bottoms used to hold the cheeses during the ripening process.

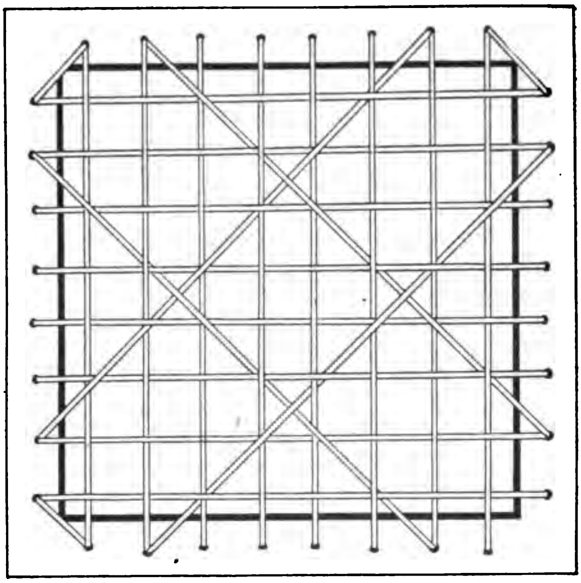

Fig. 6.—Cane bottom for ripening cheese.

First ripening room.—The first ripening room must be nearly saturated with moisture and kept at a temperature of about 60° to 62° F., as these conditions are most suitable for the proper growth of mold.

Second ripening room.—This room is to be kept somewhat cooler (56° to 60° F.), as the ripening proceeds more uniformly at this temperature. Here it is not necessary to keep such a high percentage of moisture as in the first room. There should be just enough to keep the cheeses from drying out. The walls and floors of both of these rooms should be like those of the making room—that is, easy to clean.

Both of the ripening rooms should be well ventilated and steam heated. The steam can be used not only for heating, but also for maintaining the desired degree of moisture. In summer the outside heat would raise the temperature of the rooms, causing the cheese to ripen too fast and not uniformly. For that reason some means of cooling must be provided.

PROTECTION AGAINST INSECTS.

A very important item is that of protecting the cheese against flies and other injurious insects. The outer doorways, the windows, and every other possible opening should be carefully guarded by screens with as fine a mesh as can be procured, as the smallest flies produce the most trouble. If this is not carefully attended to the cheeses are sure to become infested with fly maggots. In the ripening rooms protection against these insects can be secured to a considerable extent by keeping the rooms dark, for flies will not readily breed and multiply in a dark place.

THE MAKING OF THE CHEESE.

THE MILK.

The milk used in making Camembert cheese should be of the best quality—that is, clean and fresh. Two quarts of milk are required for each cheese.

RIPENING THE MILK.

The milk is poured into the vat and by the aid of water and steam is heated to 85° F., this being the temperature best suited for the growth of the lactic bacteria. A starter is added, the amount depending upon its strength and capacity for developing lactic acid, usually 3 quarts of a medium starter for every 100 pounds of milk. After adding the starter the milk is allowed to stand until the desired degree of acidity is reached.

This method of ripening the milk before setting is not the rule in France, where they generally set the milk at a very low degree of acidity without any attempt at previous ripening of the milk. The acid, however, develops later in the curd while the cheese is draining. In our experience serious trouble from gas has been avoided by ripening the milk before setting. Especially during the hot weather it is advisable to use a higher degree of acidity. The percentage of acidity used by us is rather high (about 0.35 per cent). This is, however, partly because of the low temperature of the room in which our experiments are made. In France the making rooms are generally kept quite warm, and the cheese will naturally drain faster there and develop the acid in the curd.

Several experiments have shown us that it is not entirely necessary to use such a high degree of acidity to secure a properly drained cheese, but by using a starter which will work rapidly after the cheese is dipped very satisfactory results have been obtained. The milk in such cases was ripened only to about 0.2 to 0.25 per cent of acid.

THE STARTER.

It is best to use a starter which is a pure culture of lactic organisms, prepared by inoculating sterilized milk with these bacteria. In cheese and butter making some homemade starter is generally used, such as sour milk or buttermilk. These often give excellent results, but are by no means pure cultures and can not be depended upon; in fact, they sometimes cause considerable trouble.

The various commercial starters have been used here and have produced excellent cheese of a mild type. The one found most satisfactory, however, was prepared from a certain brand of imported cheese. This cheese has a peculiar flavor of its own, which differs from that of any other brand. Experiments to produce this flavor have been carried out here. After many of these imported cheeses had been carefully examined and analyzed a certain kind of lactic-acid organism was found by the bacteriologist. This organism was separated, and from it a pure-culture starter prepared, which was used in the making of the cheese with excellent results. The flavor sought for has been produced repeatedly with this starter. As this brand of cheese is more popular than almost any other, this starter is probably the best that can be used in the manufacture of this cheese.[a]

ADDING THE RENNET.

The milk while ripening cools down unless carefully watched. If this has occurred, it must be brought back to the original temperature (85° F.) before adding the rennet. At this temperature it has been found necessary to use a curdling time of one and one-half to two hours to secure the texture of the curd desired for Camembert cheese. The amount of rennet required to curdle the milk in this time is calculated by means of the Marshall rennet test or the titration apparatus.

CUTTING THE CURD.

In France the method in general use consists in dipping the curd directly into the forms. Equally good results in most respects, however, have been obtained here with the curd cut. In cutting, the curd knife is passed through the curd in the vat in two directions at right angles, thus producing vertical columns of curd. When the curd has been cut in this way it drains faster, and for that reason a lower degree of acidity is used than with the curd uncut.

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[a] As soon as a demand for this starter arises in the trade, cultures of it will be furnished to such companies as regularly supply starters for other creamery work.

The most satisfactory acidity with cut curd has been found to be from 0.3 to 0.35 per cent. If it is less, the curd is likely to be too soft; if higher, the curd will drain too rapidly, will become hard and compact, and will not ripen properly. The acidity is tested as follows: A sample of milk is taken with a Babcock pipette holding 17.6 c. c. and is transferred to a glass or beaker. A few drops of phenolpthalein are added and N/10 NaOH is run in from the burette, drop by drop, until a pink color just begins to appear. The number of cubic centimeters of soda solution used, divided by 20, gives the percentage of acid in the milk.

The higher the acidity of the milk the less rennet it takes. In case the acidity is 0.3 per cent, it will take about 8 to 10 c. c. of the ordinary rennet extract to every 100 pounds of milk to bring the curd to the right consistency in one and one-half to two hours. The necessary amount of rennet is poured into a glass of water and then mixed thoroughly with the milk. The milk is now left to stand until it has coagulated to the proper consistency. It is impossible to describe any test which will show when the curd is firm enough. This can only be ascertained by practical demonstration; after a little practice the maker can generally tell just when the curd is ready to cut. The curd of Camembert cheese is much firmer than that of Cheddar or Swiss cheese.

After the curd has been cut it is stirred gently once or twice with the dipper to separate the columns and hasten the separation of the whey. Then it is allowed to stand for about fifteen minutes to make it a little firmer. The whey separates out at the surface and the bulk of it is dipped off.

If, however, the curd is quite firm, less of the loose whey is dipped off. The contents of the vat are now stirred to insure uniformity, otherwise part of the cheese would be softer than the rest.

DIPPING THE CURD INTO THE FORMS.

The next operation is the dipping. This is done with a ladle which just fits into the forms. Place the draining table near the vat, and upon it arrange the boards, each covered with a mat and holding nine of the high forms. Into each of these forms a dipperful of curd is placed, care being taken to bring the dipper inside the forms in order to prevent splashing and breaking the curd. After one dipperful is placed in each form the operation is repeated, the dipping continuing until the forms are all filled to the top.

After the curd has all been dipped into the hoops the latter are piled up, together with the boards, one upon the other. This is done partly to save space and partly to cover up the cheese and thus keep off any dirt or flies which otherwise might fall upon them. The top of the pile is then covered with an extra board. (Fig. 7.)

The curd is now allowed to drain without any artificial pressure for four or five hours. At the end of this time it will have shrunk to about half the original volume and will be ready for inoculation of molds and turning.

INOCULATION AND TURNING.

Although it is not customary for French cheese makers to inoculate Camembert cheese with mold, we have found it very desirable. Under the conditions found in Normandy the cheese acquires its moldy covering rapidly enough by accidental inoculation. Even then undesirable molds often appear to the injury of the cheese. In our experimental work artificial inoculation on the day of making has been necessary to secure satisfactory results.

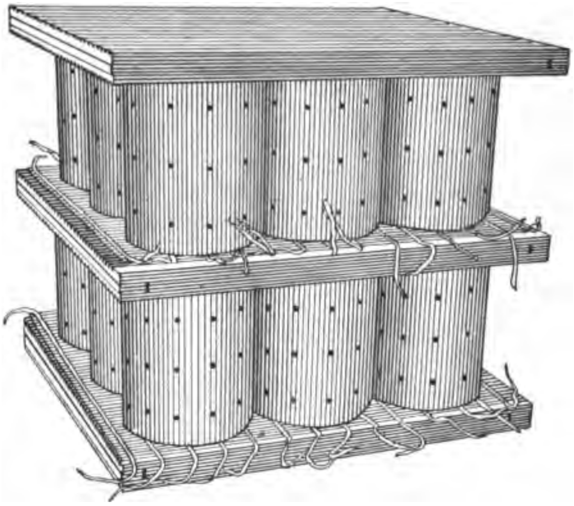

FIG. 7.—Cheese boards, mats, and forms as arranged for draining cheese.

Where dependence is placed upon accidental inoculation undesirable molds often get on the cheeses ahead of the Camembert mold, the result being either a poor cheese or one spoiled entirely. On the other hand, if a cheese is inoculated with the Camembert mold at the outset, this will grow and cover the cheese rapidly, which practically protects the cheese from the infection of other molds. A very good proof of this statement is that one can almost always find some other species of molds on imported cheese, while the molds found on inoculated cheeses are generally pure cultures, unless the culture with which they were inoculated was of poor quality. It is necessary that the maker should know the right mold when he sees it.

A most satisfactory way of inoculating is as follows: Take a small jar with a tin cover which has been punched full of small holes, like an ordinary pepper box, fill it half full with water, add a piece of moldy cracker or a piece of cheese with a good growth of the proper mold, and shake thoroughly. The contents of the jar are now sprinkled upon the surface of each cheese, then the cheeses are turned and inoculated in the same manner on the other side.

Another simple and very convenient way of inoculation, especially adapted to use in large factories, consists in taking two cheeses well covered with mold and knocking them together over the hoops. In this way enough spores drop upon the cheese to give good results.

This inoculation is by the *Penicillium camemberti;* but a second mold, *Oidium lactis*, seems to be necessary for the production of flavor in Camembert cheese, as has been indicated in a previous paper.[a] The latter is mostly found in milk and will appear on the cheese slowly. To insure its rapid growth the cheese may be inoculated with it also. The same method of inoculation may be employed as with the other mold, except that *Oidium lactis* is grown in a gelatin-culture medium instead of upon crackers.

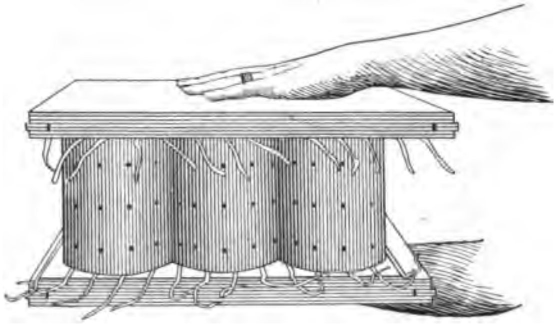

FIG. 8.—Method of turning cheese.

The cheeses are turned, not only to secure the inoculation of both sides, but also to prevent them from becoming too compact on the underside on account of the greater pressure there and to insure a smoother surface on both sides. The quickest and easiest way to turn the cheeses is to cover the nine forms with a second mat and board. Place one hand under the lower board and the other over the upper, and then invert. (Fig. 8.) If the cheeses thus turned do not rest flat on the bottom they are straightened out by moving the forms.

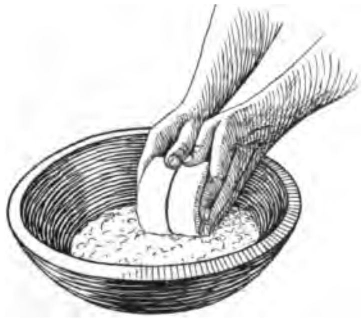

FIG. 9.—Salting the cheese.

After turning and inoculating, the cheeses are left without any further handling until the next morning, when they are taken out of the forms and salted. By this time they have shrunk almost to their final size. In case they are not yet hard enough to be safely handled, they are turned again and left to stand until they are sufficiently firm.

SALTING.

The salting is done by taking two cheeses together and rolling the edges and rubbing their surfaces in salt. (Fig. 9.) The salt to be used should not be too fine, as this would produce oversalting.

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[a] Bulletin No. 82, Bureau of Animal Industry.

After salting, the cheeses are placed upon dry boards, so that the sides which were previously at the top will now be at the bottom. The next morning it will be found that all of the salt has dissolved, and that most of it is diffused in the cheese. The cheeses are again transferred to another dry board or cane bottom, after turning, and are ready for the ripening process. The reason for transferring them to dry boards is that a dry board is less apt to become covered with mold.

MAKING CHEESE FROM UNCUT CURD.

A cheese from uncut curd is made somewhat differently. Although the cut curd drains more rapidly, the draining of the uncut curd can be greatly facilitated by allowing the milk to become more acid before adding the rennet.

In our experiments the degree of acidity giving the most satisfaction in the uncut curd has been about 0.40 per cent. The amount of rennet to be added varies inversely as the acidity. When the curd has reached the proper consistency, it is dipped into the hoops in the same way as the cut curd, but the operation should be carried out more slowly. After the forms have been filled the cheeses are allowed to stand without turning until the next morning. This is because the successive dipperfuls of uncut curd do not stick together readily at first and must be given more time.

While turning the cheese the next morning they are to be inoculated. They must then be left until the following morning, by which time they are ready to be salted. After salting they remain another day in the making room, making three days altogether, instead of two as in the case of the cut-curd cheese.

In France the cheeses are always made of uncut curd, but no reason has ever been given for the practice, so far as the writer knows. In a series of experiments where cheeses were made of the same milk with cut as well as uncut curd for comparison we found that in almost every case the uncut-curd cheese, even when fully ripe, did not decompose as quickly as the cut-curd cheese. Other advantages are that more cheese is produced from the uncut curd from the same amount of milk, and the loss of fat in the whey is not so great.

THE USE OF THE LOW FORMS.

Both cut and uncut curd cheeses should be hard enough to bear handling at the time of salting, but often they are not yet hard enough to retain their shape. In such cases they should be put at the time of salting into the low forms, where they remain until the next morning. When they can hold their shape without the aid of the forms they are taken to the ripening room.

RIPENING THE CHEESE.

The cheeses are removed to the first ripening room. Here they are placed on smooth boards upon shelves. The boards are of the same size as the draining boards, but have a smooth surface. The cheeses remain on these boards during the whole ripening period. Cane bottoms are frequently used and are preferable to the boards for the following reasons: When boards are used the molds are apt to grow into the wood, causing the latter to stick so tenaciously that on turning the cheeses over the rind is torn off. On the other hand, when cane bottoms are used the mold can grow more uniformly on both sides of the cheeses, and as they do not stick to the bottoms so tenaciously, it is necessary to turn them but once or twice in the first room, which reduces the labor considerably. The cheeses resting on boards must be turned daily.

During the first week any ripening which occurs is not noticeable, and the cheese remains in the form of hard curd. The surface of the cheese often becomes slightly slimy, and some change in the color can be noticed. Toward the end of this first week the mold can be seen upon looking closely.

During the second week the mold, when once started, grows very rapidly; and in the course of one or two days it covers the cheese completely, giving it a snow-white, cotton-like appearance. This white coat of mold turns to a gray green within two to four days, and by this time the cheese begin to show actual ripening. The cheese first becomes soft just under the coat of mold, and the ripening proceeds gradually toward the center. On cutting the cheese open a thin layer of softened curd can be observed under the mold. The texture of this ripened part is creamy and soft, just as the whole cheese will be at the time of complete ripening.

If the cheeses remain upon the shelves in the ripening room under proper conditions, as they often do in France, they will ripen completely. But under our conditions, where the air is dryer, we have found it necessary to wrap the cheeses during the second week in parchment paper or tin foil. This prevents evaporation and hardening, checks the growth of mold, and promotes the growth of the other organisms, thus hastening the ripening. When the cheeses appear dry and tend to become hard, tin foil seems to give the better result, but in the factories in the trade parchment paper is nearly always used. The cheeses wrapped in tin foil very commonly develop stronger flavors and softer texture than those wrapped in paper. The time of wrapping affects the kind of cheese produced, and the intensity of the flavor can be partly regulated in this way. If a cheese with a strong flavor is desired, the wrapping must be done when the cheese is only slightly covered with the white mold. The wrapping checks the growth of the latter and promotes a more rapid development of the other mold, *Oidium lactis*. On the other hand, a mild flavor can be obtained by wrapping the cheese after the growth of mold has become luxuriant and has turned blue.

After being wrapped the cheeses are often put in small, round boxes, which they fit tightly and in which they are later shipped to market. These boxes help to maintain the shape of the cheeses, which become quite soft during ripening. At the end of the second week the cheeses are transferred to the second ripening room, where they remain until they are ready for shipment, or, if desired, until they are fully ripe. During the third week the ripening proceeds rapidly, and the cheeses become one-half to two-thirds ripe. On the surface slimy, reddish spots appear, and the cheese begins to give off a characteristic Camembert odor. Between the third and the fourth week the hard curd in the center usually disappears, and the cheese has a creamy, waxlike texture. The delicious flavor found in all Camembert cheeses is now evident. A little hard curd may still be found in the center of the cheese, but this will disappear if given time.

FACTORY METHODS.

In factory practice in France and also where these cheeses are now made in America they are wrapped and put into boxes as soon as the covering of mold is well started. This is when they are about two weeks old. Instead of ripening further in the factory, they commonly are sent to market at once. Further ripening thus becomes a matter for the dealer. Although this is the common practice in France, some factories ripen the cheese quite fully to supply a special trade. In other cases dealers establish cellars, where the cheeses are taken out of the boxes, are unwrapped, and are ripened completely on shelves before selling. Others allow them to ripen as they may in the boxes. It seems desirable to recommend that where domestic factories are supplying our own market, cheeses be ripened far enough to guarantee good results before they are sent out of the factory.

VARIOUS DEFECTS OF CHEESE.

Gassy curd.—In the making of Camembert cheese, as in making any other kind, numerous difficulties are encountered. One of the most common troubles is that arising from gassy curd. (Fig. 10.) In this case the fault generally lies in the milk, being due to gas-producing bacteria. No way has been found in which this difficulty can be absolutely avoided, but it may be partly remedied by increasing the amount of good lactic starter and the development of higher acidity before setting, which will in time overpower the gas-producing organisms. If the curd is kept at a low temperature after dipping, the growth of these gassy organisms is checked to some extent. The gas can not always be detected in the fresh curd, but sometimes develops later, and if it does the cheese very seldom turns out satisfactorily.

Yeast.—Another difficulty is caused by yeast. The cheeses often become covered with yeast in the making room, although sometimes the yeast makes its appearance after the cheeses have been taken to the ripening room. The surface of such cheeses becomes slimy and sticky, causing the cheeses to stick to the boards, so that when they are turned a thin skin is torn off. In such cases it is difficult to obtain a good growth of mold, for the latter is pulled off with the thin film of yeast, the cheese does not ripen properly, and it often has a strong, bad flavor.

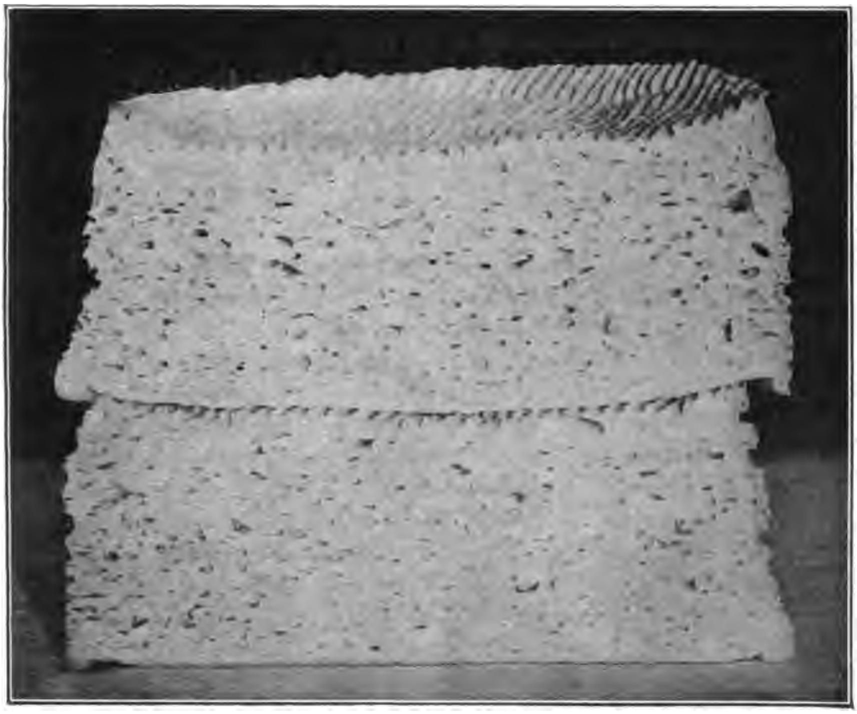

FIG. 10.—Gassy curd.

Molds.—Contamination from the other varieties of mold causes considerable trouble. If the cheeses contain spots of green or brown mold, or if a long, fuzzy mold, sometimes with black tops (Mucors), appears, the Camembert mold can not grow properly, and the result is often a bitter cheese or one with other undesirable flavors. The Camembert mold will sometimes grow over and cover the green and other molds, but this does not prevent them from producing an objectionable flavor.

When such infection from foreign molds occurs, the whole equipment should be sterilized, and if possible the walls and floors of the making as well as the ripening rooms should be cleaned and whitewashed.

Dry cheese.—The drying out of cheese is caused by lack of moisture in the ripening rooms, or by too rapid draining of the curd. Such cheeses can often be saved, if the drying out has not proceeded too far, by wrapping them tightly in tin foil.

Wet cheese.—A defect just the opposite of the last is found in wet cheeses. It is caused by too low a temperature of the making room, as well as by too low a degree of acidity of the milk, both of which retard the draining of the cheese. It may also be caused by too high a degree of moisture in the ripening rooms.

The ripening of such cheeses is more in the nature of a liquefaction, and the interior becomes so soft that it would run out if the cheese were not kept in a box. There is no hope for such cheeses, as the flavor and texture will never be satisfactory.

Mites.—Serious damage is done to cheeses by the cheese mite, a small insect scarcely visible to the naked eye. These mites crawl all over the cheese and eat up or destroy the mold, so that the cheese will not ripen properly and is practically ruined. The only remedy in such cases is the thorough disinfection of the whole plant.

Skippers.—Another enemy of the cheese is the cheese skipper—the larva of a small fly. The flies lay their eggs on the cheese, and these hatch out in a short time. The skippers remain on the surface and can be scraped off, but not without spoiling the appearance of the cheese and possibly leaving unhatched eggs. Such cheeses can not be sold and are practically lost.

ESTIMATED EQUIPMENT FOR A FACTORY.

The estimated equipment for a factory using about 1,000 pounds of milk per day is indicated below. Before building such a plant, however, it is always desirable to visit some dairy establishment where the essential equipment would be as nearly comparable to that needed as possible. This need not necessarily be a Camembert-cheese factory. Any properly equipped dairy establishment will give ideas as to the arrangement of steam and water pipes, vats, etc.

In addition to this ordinary creamery equipment a Camembert-cheese factory requires its own special apparatus.

Calculated for 1,000 pounds of milk, which will produce 250 cheeses, this will require for the making room:

250 high hoops.
500 low hoops.
150 draining boards (if used in making room only).
150 mats.
Draining table to accommodate 250 cheeses (42 square feet of surface).
Shelf room enough to accommodate 250 cheeses on the second day of draining.

Vats and draining tables should be so arranged as to minimize the labor of dipping. The two ripening rooms must be large enough to accommodate the entire output for about twenty days, i. e., 5,000 cheeses. If the cheeses are kept on boards such as are used in the making room, this would require about 500 boards in constant use. These would occupy 700 running feet of shelving. The shelves should be about 5 inches apart. A rough calculation will show that a total curing space of 14 by 14 by 8 feet would be large enough to accommodate all the cheeses. The arrangement of shelving is a matter of economical utilization of all the available space. Aisles between the shelves should be at least 3 feet wide to give sufficient room to do the necessary work. It probably would require a maker and one helper to run such a factory.

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Issued May 11, 1907.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN No. 99.

A. D. MELVIN, CHIEF OF BUREAU.

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THE DANGER FROM TUBERCLE BACILLI IN THE ENVIRONMENT OF TUBERCULOUS CATTLE.

BY

E. C. SCHROEDER, M. D. V.,
Superintendent of Experiment Station,

AND

W. E. COTTON,
Expert Assistant at Experiment Station

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., April 4, 1907.

Sir: I have the honor to transmit herewith, and to recommend for publication as Bulletin No. 99 of this Bureau, an article entitled "The Danger from Tubercle Bacilli in the Environment of Tuberculous Cattle," by Dr. E. C. Schroeder and W. E. Cotton, of the Bureau Experiment Station.

The investigations with which the article deals show that tuberculous cattle, before they lose their general appearance of health, often pass feces heavily infected with tubercle bacilli, and that the same is true of healthy cattle that are permitted to swallow tubercle bacilli in their feed or in their drinking water. When test animals are inoculated with such feces, or with milk soiled with such feces, they commonly become affected with tuberculosis.

The work as a whole shows that the general condition or appearance of a tuberculous animal gives no indication as to the time when it will begin to distribute tubercle bacilli and become dangerous; that the milk from all tuberculous cattle, irrespective of the condition of their udders, should be regarded as dangerous, and that even the milk of healthy cows if it is drawn in the environment of tuberculous cattle may contain tubercle bacilli.

Since the tuberculin test is the only practical means of detecting tuberculosis in the live animal before an advanced stage is reached, the importance of testing dairy cows with tuberculin and removing every reacting animal is again emphasized.

Respectfully,
A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

	Page.
Introduction	7
Description of cattle used in experiments	8
Examination of feces for tubercle bacilli	8
Microscopic examinations	8
Inoculation tests with guinea pigs	9
Summary of feces examinations	11
Tests of saliva, nasal discharge, urine, and milk	11
Feces in milk the real danger from tuberculous cattle	13
Persons probably contract tuberculosis chiefly by ingestion	17
Infectiousness of soiled milk shown by inoculation of guinea pigs	18
Additional feeding and inoculation tests	21
Conclusions	22

THE DANGER FROM TUBERCLE BACILLI IN THE ENVIRONMENT OF TUBERCULOUS CATTLE.

INTRODUCTION.

The manner in which tubercle bacilli are thrown out by and reach the environment of persons affected with tuberculosis has been carefully studied; consequently, we know with practical certainty that all cases of human tuberculosis may be divided into two classes—" open," or dangerous, and " closed," or less dangerous. A " closed " may at any time become an " open " tuberculosis, but it is from the latter only, which includes all persons whose lungs, air passages and immediately related structures are affected, that tubercule bacilli are disseminated in a way dangerous to health. This is the view sanctioned by Dr. Robert Koch in his " Nobel lecture," delivered at Stockholm on December 10, 1905.[a]

Concerning tuberculous animals our knowledge is not so definite, but it is of the highest importance that we should know how tubercle bacilli are scattered by them, too, not that we may establish an " open " and a " closed " class, but to enable us to know how we can best prevent the transmission of tuberculosis from animal to animal and from animal to man.

Many prominent investigators are convinced that tuberculosis of animals constitutes a serious danger for man; others are not; but all agree that it is an occasional danger, more frequently for children, whose diet consists largely of milk, than for adults. While this matter awaits a solution and there is not perfect harmony among authorities, we may reasonably assume that it is better to guard against exposure to infectious material scattered by persons and animals than only against that scattered by persons. Protective measures that take into account the clearly defined menace and neglect the highly probable danger are insufficient, especially when the probable danger is, as in this case, believed to be actual by many men who are competent to judge.

But even if persons are eventually shown to be comparatively safe against infection from tuberculous animals, the manner of dissemination is nevertheless an important economic problem, because animals have been repeatedly shown to be more susceptible to tuberculous infection from animals than from man, and tuberculosis among animals is, from the stock owner's point of view, one of the very serious conditions with which the animal industry has to deal.

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[a] Translation in the Lancet of May 26, 1906.

In this article are recorded some observations on the tubercle bacilli expelled by cattle, and an attempt is made to show how these bacilli may be scattered and become a great danger to animals, and, as we believe, also to man.

DESCRIPTION OF CATTLE USED IN EXPERIMENTS.

The cattle from which the material was obtained for the observations require the following short description:

Cow No. 1 has been affected with tuberculosis for several years. Aside from a tuberculin test, which showed her to be tuberculous, there is nothing in her condition that would condemn her for use as a dairy cow.

Cow No. 113 is affected with advanced tuberculosis. She is still alive, quite thin, and has a severe and frequent cough.

Cows. Nos. 84, 374, and 384 are three healthy animals that were fed a small amount of culture of tubercle bacilli daily in their drinking water during the time the observations were in progress.

Cow No. 325 and steer No. 329 are two cases of recent tuberculosis; they are living, and the knowledge that they are tuberculous is based entirely on a tuberculin test.

Cow No. 372 was found on autopsy to have a tuberculous affection limited to one post-pharyngeal lymph gland.

Cow No. 373 was found on autopsy to be affected with tuberculosis of the lung and liver and the mediastinal, portal, and mesenteric lymph glands. The lesions were widely scattered, but not extensive.

Cow No. 476 is living, is in good condition, and was used until recently, and would be in use now, as a private family cow, if a tuberculin test had not shown that she is affected with tuberculosis.

EXAMINATION OF FECES FOR TUBERCLE BACILLI.

MICROSCOPIC EXAMINATIONS.

Small portions of feces, taken at random from the interior of masses freshly passed by the cattle, were smeared on thin squares of glass, which are generally known in connection with microscopic work as cover-glasses. Similar preparations were made with material gently scraped from the mucous surface, just inside the anal orifice, of the rectum. The cover-glasses were dried, stained, and examined microscopically. Those made with material from the following cattle were found to contain bacteria identical in appearance with tubercle bacilli: Nos. 1, 84, 113, 325, 373, 374, and 476.

The number of bacilli per cover-glass varied with different cattle, and on different days with the same animal, from 0 to 25, and were usually a little more numerous in the rectal scrapings than in the feces. In some cover-glasses made with shreds of mucus picked from the feces the bacilli were much more numerous and in some instances innumerable.

Cover-glass preparations similarly made with feces and rectal scrapings from nontuberculous cattle were found to be free from anything resembling tubercle bacilli.

With a simple microscopic examination it is difficult to distinguish between tubercle bacilli and some harmless micro-organisms known as acid-fast bacteria. At first it was believed that the bacilli in the preparations belonged in whole or in part to the latter group, but this view was not tenable after it was discovered that similar bacilli could not be found in material from nontuberculous cattle.

INOCULATION TESTS WITH GUINEA PIGS.

To establish absolutely that the bacilli were virulent tubercle germs, a series of inoculation tests with small animals was undertaken.

Two lots of guinea pigs were inoculated from each of the following cattle: Nos. 1, 84, 113, 325, 373, 374, and 384, one lot with feces and the other with scrapings from the rectal mucosa. Cattle Nos. 329 and 372 were omitted from the inoculation experiments, as they had given negative results with microscopic examinations, and cow No. 476 was received too late to be included.

The guinea pigs were inoculated under the skin on the inside of the right thigh; each received an amount of material equivalent in weight to about one-eighth of a grain, and more than 25 per cent of them became affected with septicemia and died before a tuberculous affection had time to develop. The results obtained with the animals that lived long enough to contract tuberculosis are as follows:

Cow No. 1.—Guinea pigs inoculated April 28, 1906, with feces did not become tuberculous; those inoculated on the same day with scrapings from the rectal mucosa were found on post-mortem examination to be affected with tuberculosis. Guinea pigs inoculated June 19, 1906, with feces, as well as those inoculated with scrapings from the rectal mucosa, were found on post-mortem examination to be affected with tuberculosis. Guinea pigs inoculated June 27, 1906, with feces, as well as those with scrapings from the rectal mucosa, remained free from tuberculosis.

We have from this cow one positive and two negative results with feces and two positive and one negative with scrapings from the rectal mucosa. It should be borne in mind that the subcutaneous inoculation of guinea pigs with cattle feces can not be regarded as a delicate test for the presence of tubercle bacilli, and this is to nearly the same extent true of material scraped from the rectal mucosa, which is composed largely of feces. Cattle feces are a complex substance relative to the number and different kinds of bacteria contained. Each inoculation is followed by a local inflammation and suppuration, and hence the tubercle bacilli that are present, if their number is few, have every chance to be overcome by more rapidly growing organisms. The pathological conditions produced by the latter may in themselves be sufficient to prevent the tubercle bacilli from establishing themselves. That the acid-fast bacteria observed in the feces of cow No. 1 are certainly infectious tubercle bacilli is demonstrated absolutely by the occurrence of tuberculosis among the guinea pigs. That the feces and the scrapings from the rectal mucosa inoculated into guinea pigs that remained free from tuberculosis did not contain infectious tubercle bacilli can not be asserted positively. It is quite likely that the bacilli were present but did not have a chance to get actually into the tissues, and this view is supported by the fact that acid-fast bacteria were shown to be present in the feces of the cow on each of the three days on which the inoculations were made.

Cow No. 84.—Guinea pigs inoculated May 2, 1906, with feces did not become tuberculous; those inoculated with scrapings from the rectal mucosa were found on post-mortem examination to be affected with tuberculosis. Guinea pigs inoculated on other days from this cow died affected with septicemia before tuberculosis had time to develop.

Cow No. 113.—Guinea pigs inoculated with either feces or scrapings from the rectal mucosa on April 27, 1906; June 19, 1906, and June 27, 1906, were all found on post-mortem examination to be affected with tuberculosis.

Cows Nos. 325, 373, 374, and 384.—Guinea pigs inoculated with feces and scrapings from the rectal mucosa either died from septicemia before tuberculosis had time to develop or were found to be free from lesions of tuberculosis on post-mortem examination.

It was shown in another experiment[a] that the acid-fast bacteria microscopically demonstrated in the feces of cows Nos. 374 and 384 were virulent tubercle bacilli. The feces in the experiment referred to were fed to four hogs with the result that three of them became affected with tuberculosis.

The reasons why the inoculation of guinea pigs with feces is not a delicate test for the presence of tubercle bacilli do not apply when the feces are swallowed. There is no inflammation or suppuration in the intestine caused by swallowing large numbers of bacteria of the kind ordinarily contained in the feces of cattle. Bacteria, excepting those that are causative agents of specific diseases, if they pass through the uninjured wall of the stomach or bowels, are rapidly destroyed and cause no marked objectionable condition and no determinable pathological lesions. That tubercle bacilli, on the other hand, may pass through the uninjured wall of the intestine, without causing local lesions, is claimed on the basis of experimental evidence.

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[a] See Bulletin 88, Bureau of Animal Industry.

SUMMARY OF FECES EXAMINATIONS.

To sum up the results as far as we have gone, among seven natural cases of tuberculosis, we have five, or 71.43 per cent, that were found on microscopic examination to be passing tubercle bacilli in their feces, and the two cattle that failed to show bacilli were a cow with an affection, strictly "closed," limited to one post-pharyngeal gland, and a young steer in which the presence of tuberculosis would not have been suspected without a tuberculin test.

The feces of four of the five cattle were used in inoculation tests and were found to be infectious in two cases. To these a third must be added, as subsequent inoculation tests hereinafter described will show.

Three cows that were fed culture of tubercle bacilli showed the presence of the bacilli in their feces on microscopic examination, and that the feces were infectious is shown for one cow by guinea pig inoculations and for the other two by the hogs that were fed with their feces in an earlier experiment. With this evidence we can not doubt that the tubercle bacilli that are swallowed by cattle pass in part through the entire length of the digestive tract and out through the rectum without losing their infectiousness.

Animals do not expectorate. The infectious material that is expectorated by tuberculous persons has its counterpart in tuberculous animals, but it is swallowed and not thrown out as sputum. The absence of real cavity formation in the lungs of cattle, such as occurs in tuberculous human lungs, does not seem to militate against the number of bacilli that may pass into the environment of tuberculous cattle. It must not be supposed that the occurrence of the bacilli in the feces of the cattle affected with naturally acquired tuberculosis was due to intestinal disease, as tuberculosis of the intestinal mucosa of cattle is an extremely rare affection.

TESTS OF SALIVA, NASAL DISCHARGE, URINE, AND MILK.

The saliva, nasal discharge, and urine of two cows—Nos. 1 and 113—and the milk of three cows—Nos. 84, 372, and 373—were tested for tubercle bacilli by microscopic examination and guinea-pig inoculation, and the milk further by ingestion experiments.

The microscopic examinations were all negative,[a] and the inoculations were likewise negative, with the exception of the saliva of cow No. 113, which invariably produced tuberculosis. The milk was fed to a large number of guinea pigs for long periods of time, and other guinea pigs were injected with it, intra-abdominally, from time to time, without causing disease of any kind.

The urine of the cows was drawn from their bladders through a catheter with antiseptic precautions and placed for thirty minutes in the tubes of a small electrical centrifugal machine that made 2,000 revolutions per minute. As the specific gravity of tubercle bacilli is comparatively high, had any been present in the urine this treatement would have thrown them to the bottom of the tubes. Only the lower third of the urine in each tube was used for making intra-abdominal injections, and each guinea pig injected received a dose of 5 cubic centimeters.

The microscopic and inoculation tests with saliva, nasal discharge, and urine from cows Nos. 1 and 113 were twice repeated, giving three tests of each kind of material, each test on a different day. The milk from cows Nos. 84, 372, and 373 was removed from their udders with proper care to prevent its contamination with feces.

The absence of tubercle bacilli from the urine of cows Nos. 1 and 113, especially from that of the latter, indicates that they are rarely or never passed in the urine unless the genito-urinary organs are affected. The condition of the two cows relative to that of the other cattle strengthens this inference, as a larger number of positive results were obtained with the inoculation of their feces and scrapings from their rectal mucosa than with similar material from other cattle; and, further, the saliva of one of them (No. 113) infected every guinea pig inoculated with it. But neither the urine tests nor the cattle were sufficient in number for fully reliable negative conclusions, and this subject will receive further attention in the future if material for additional investigations can be obtained.

It was a source of surprise that no positive results followed the inoculations with nasal discharge, because the location of the nasal chambers seems well adapted for their frequent infection with material expelled from the lung. Cow No. 113 suffers with frequent severe paroxysms of coughing, during which her mouth is open and the air is largely, if not wholly, impelled through it. This may account for the presence of tubercle bacilli in her saliva and their absence from her nasal discharge.

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[a] Subsequent microscopic examinations of the saliva of cow No. 113, in cover-glasses made from the sediment in tubes in which it was mixed with normal salt solution and then centrifugalized, showed the presence of tubercle bacilli.

Cow No. 1 is much less severely affected than No. 113, and the absence of positive results from inoculations with her saliva and nasal discharge is attributed to the infrequency and mildness with which she coughs. Her cough is rarely more than a gentle effort to clear her throat, and any substance expelled from her lung would barely pass her fauces and would be swallowed without reaching the forward portions of her mouth. The regurgitation and remastication of food that is practiced naturally by ruminants would tend to clear her throat as much as to infect it with swallowed infectious material. The infectious material expelled from the lung and swallowed does not necessarily lodge in the rumen, from which regurgitation takes place, and certainly does not become as thoroughly and evenly mixed with the contents of the rumen as it is with the feces.

The absence of positive results from the inoculations and feeding tests with milk is in perfect harmony with the writers' past experiences with milk from tuberculous cows with healthy udders when the milking is done with proper care against the introduction of infectious material that is not associated with the interior of the udder or the milk-secreting structures. The danger that milk may become infected from the environment of tuberculous cows is so great that we are justified in asserting that the few positive results we have had during many years with intra-abdominal injections of guinea pigs with milk from tuberculous cows with unaffected udders were not due to tubercle bacilli that were drawn with the milk, but to tubercle bacilli that dropped into it or into the milk pail from the exterior of the cow, despite all precautions to the contrary. Since it does not seem reasonable for tubercle bacilli to leave the body with the urine unless the genito-urinary apparatus is affected, similarly it does not seem reasonable for them to pass out with the milk through a healthy udder.

FECES IN MILK THE REAL DANGER FROM TUBERCULOUS CATTLE.

The results of the tests point to the conclusion that the real danger from tuberculous cattle lies in the manner in which tubercle bacilli are disseminated with their feces.

The average number of acid-fast bacilli found in the feces of tuberculous cattle—leaving the greater number in the rectal scrapings and selected shreds of mucus out of consideration—is 6 per cover-glass. That these bacteria are tubercle bacilli has been sufficiently demonstrated by the positive results obtained with the inoculation tests and by their absence from the cover-glasses prepared with the feces of nontuberculous cattle. Now, let us calculate what this signifies as to the amount of infection that may be scattered by a single tuberculous cow in one day.

The cover-glasses were carefully weighed on a delicate balance before and after spreading a layer of feces on them. The amount of moist feces per cover-glass was found to have a maximum weight of one-thirtieth of a grain; it was usually less, and frequently not more than one-half as much. When one of the prepared cover-glasses is examined with the microscope the first condition noticed is that only a portion of it, rarely one-half, is sufficiently transparent for the detection of tubercle bacilli. It is reasonable to assume that the thicker, opaque portions of the film of feces are heavier and contain a larger number of bacilli than the lighter, transparent portions; hence, the tubercle bacilli that come into view after prolonged search are less than half the number actually present. To one who is familiar with microscopic work it would not appear unreasonable if we made the flat assertion that it is impossible to find more than 10 per cent of the tubercle germs that are present in cover-glasses of the kind we made for our examinations. But it is not necessary to present the fact in this extreme. If we take only the actual average number of tubercle bacilli seen, and the maximum weight of feces per cover-glass, we have 6 bacilli in one-thirtieth of a grain of feces, and we can not avoid feeling amazed at the amount of infection which this represents as leaving the body of a tuberculous cow daily.

A cow of average size passes about 30 pounds of moist feces each day, and if the whole of this mass could be spread on cover-glasses similar to our preparation, it would be sufficient to make 6,300,000 preparations, which would contain 37,800,000 microscopically demonstrable tubercle bacilli.

It is a significant fact that our microscopic examinations indicate that the bacilli in the feces, excepting those in the shreds and masses of mucus that do not enter into the present calculation, are evenly distributed, so that we do not have some portions of the feces that are very infectious and others that are innocuous. Practically it is all infectious, and every part of it is dangerous, and the mucus shreds alone show extreme infectiousness. The even distribution of the bacilli likewise indicates that they entered the intestine at the upper end of the digestive tract, as a considerable amount of churning, such as the food receives in the stomach and intestines, is required to effect this distribution.

Tuberculous persons from whom tubercle bacilli are being disseminated can be taught to use various precautions that will reduce to a minimum the danger to health in their environment. Sputa can be expectorated into receptacles containing germicidal fluids, and a cloth can be held before the mouth during paroxysms of coughing and at once treated so as to destroy the infectious material that is impelled against or into it. Such persons can have individual sleeping apartments, and they should be prevented from coming into contact with articles of diet that are used by healthy persons. Their own individual generosity, when they are informed of their condition and its danger to health, will with rare exceptions induce them to take many precautions for the safety of their families and associates. Healthy persons, when they are informed of the danger involved in close contact with those affected with tuberculosis, and know that a chronic cough must be regarded as a very suspicious circumstance, can do much for their own protection by avoiding association on terms of close intimacy with persons who are actually or probably tuberculous.

With cattle we have a different state of affairs. Effective germicidal substances are too expensive and their proper application to large masses of feces daily is too difficult and troublesome for practical purposes. Feces are dropped everywhere in the environment of cattle—in stables, fields, and barnyards, and on roadways. They are splashed on the bodies of cattle, and frequently cattle lie down and get their bodies coated with them, and this is especially true of the parts of the body close to the udder. Feces are thrown against partitions of stalls and walls of stables and are promiscuously switched about by the soiled tails of animals, and no precaution or measure of cleanliness can entirely prevent them from getting into the milk pail occasionally and on the hands and clothing of the stable attendants and milkers frequently. We have seen large quantities of milk strained in many dairies, but have not found the dairy in which the milk was removed from the cows with a degree of cleanliness so perfect that the cloth or screen through which it was strained did not show the presence of some cattle hairs and fragments of a substance suspiciously like feces. In some dairies the quantity of feces that enters the milk pail, both fresh and dry, to judge from that which collects in the strainer through which it is poured, is comparatively large. The precautionary measures that can be used by the dairyman are limited by the price of milk. He can not afford to use measures the cost of which is so great that their application would convert his business into a philanthropic enterprise.

Regarding the dairy industry, we know the two following important facts: (1) That the commonest disease with which cows are affected is tuberculosis, and (2) that milk in some form reaches practically all persons. The person who does not use milk, cream, butter, or cheese is a rare exception to a general rule, and the family that uses no fresh dairy product can hardly be said to exist.

When we know how completely cattle feces may be charged with tubercle bacilli and how easily milk may be infected from this source, and contemplate this fact, keeping in mind the wide distribution that dairy products have, and add to our knowledge some of the results recently obtained and published by competent investigators, we must conclude that the eradication of tuberculosis among cattle can not be too vigorously urged or pursued.

Our own investigations have shown that pulmonary tuberculosis is the most common form of the disease in animals, irrespective of the point at which the tuberculous infection enters the body, and that tubercle bacilli may pass through the intestinal wall and reach the lung without causing visible disease of the intestinal mucosa.

Nicholas and Descos and Ravenel proved by feeding healthy dogs on tuberculous fluids and examining the chyle in the thoracic duct a few hours later that tubercle bacilli may readily pass through the intestinal wall and infect the animal without causing lesions in the intestines.

For the benefit of readers who are not informed on the subject of anatomy it may be well to say that the thoracic duct is the common trunk of all the lymphatic vessels that drain the abdominal cavity and the organs contained in it, and an additional large portion of the body, and that it discharges its contents into the anterior vena cava, one of the large veins in which the blood is returned to the heart. The material discharged by the duct into the vein is carried with the blood directly to the heart and pumped by the heart to the lung, where it is filtered through the exceedingly fine capillary network in which the blood is arterialized. It can readily be seen that tubercle bacilli that have passed through the intestinal wall and through the lymphatics into the thoracic duct have a clear way to reach and infect the lung.

Vallée calls attention to the fact that the lung is the favorable location of the tubercle bacillus in all species of animals, and he made experiments that indicate that infection through the digestive tract constitutes a mode of inoculation which is extremely favorable to the production of pulmonary tuberculosis. He concludes that ingestion is the quickest and most certain method for the tubercularization of the lymph glands associated with the lung, and that the tubercle bacillus may pass through the intestinal wall without producing appreciable lesions in the mucous membrane of the intestine or in the mesenteric lymph glands.

Schlossman and St. Engle found that tubercle bacilli introduced into the stomachs of guinea pigs by means of a laparotomy could be found in the lungs in a few hours. Calmette produced pulmonary tuberculosis of a goat by introducing suspensions of tubercle bacilli into its rumen. Cadéac asserts after careful investigation that the widely accepted hypothesis of the transmission of tuberculosis by inhalation of dust from dried sputa has not been proven; and he showed that sputum dries slowly, is difficult to pulverize, and rapidly loses its infectious character.

PERSONS PROBABLY CONTRACT TUBERCULOSIS CHIEFLY BY INGESTION.

It has long been supposed that the frequency with which tuberculosis occurs among persons is due to the direct infection of the lung (probably because the lung is most frequently affected) with sputum which has become dried and pulverized after being expectorated by persons affected with tuberculosis. This supposition or theory is gradually being shown to be erroneous, (1) because sputum does not pulverize easily and loses its infectious character in a short time; (2) because the greater frequency with which the lung is affected as compared with any other organ of the body is accounted for in a way that does not require the acceptance of the theory of direct infection from the air, and (3) because it is being shown by a number of investigators that tubercle bacilli may pass through the intestinal wall without injuring it and thus may reach the lung, and that the ingestion of tubercle bacilli is the quickest and simplest way to infect the lung. In other words, the inhalation of tubercle bacilli is losing much of its importance in the minds of investigators, and the swallowing of tubercle bacilli is gradually supplanting it as the true mode of infection. It therefore becomes necessary to give the most careful attention to the sources from which tubercle bacilli may reach our daily food and drink.

There is little doubt that many persons become affected with tuberculosis by breathing tubercle bacilli in an infected environment. Infectious material sprayed from the mouths of tuberculous persons lodges in the upper air passages, is drawn or drops into the mouth, and is swallowed and taken up by the intestines, and through the lymph channels reaches the lung. Other cases result from tuberculous persons who are allowed to handle and prepare food and to clean and care for kitchen and table utensils.

But there is little doubt in the writers' minds that tubercle bacilli from no source receive a wider distribution in a perfectly fresh state than those that enter the milk with fragments of feces from tuberculous cows. As we have already pointed out, tuberculosis is the most frequent disease with which dairy herds are affected, and milk has an enormously wide distribution. It is taken everywhere on every day and reaches practically every person on every day.

INFECTIOUSNESS OF SOILED MILK SHOWN BY INOCULATION OF GUINEA PIGS.

These facts make it desirable to know something definite about the significance of the frequently unavoidable introduction of feces into milk, and we have tried to gain light on the subject by inoculating guinea pigs with milk into which small fragments of feces from tuberculous cows were placed in a manner as nearly as possible like that which occurs in a dairy stable.

Normal, fresh milk from healthy cows was soiled with small masses of feces from cows Nos. 1, 84, 113, 325, and 373. The amount introduced into each sample of milk was very small, about as much as would enter in proportion to the volume of milk in a dairy stable in which average cleanliness is practiced. Some of the soiled milk was injected without further treatment and some was strained through linen cloth and then injected. The injections were intra-abdominal, and each guinea pig received a dose of 5 cubic centimeters.

To make sure that the milk used in this experiment was free from infectious material before it was soiled with the feces, a number of guinea pigs were injected with it from time to time in its pure unsoiled state. Such guinea pigs remained well until they were killed, and on post-mortem examination were found to be free from lesions of disease.

The following tables give the results obtained with the injections of soiled milk. No table is given for cow No. 325, as no tuberculosis was caused by the milk soiled with her feces. In all other respects the inoculations were identical with those of cow No. 373.

Results of injecting guinea pigs with normal milk (from healthy cows) soiled with feces from cow No. 1 (tuberculous).

MILK NOT STRAINED.

No. of guinea pig.	Date of injection.	Date of death.[a]	Remarks.
7608	June 19, 1906	July 24, 1906	Tuberculosis of liver, spleen, and abdominal lymph glands.
7609	.....do.......	.....do.......	No lesions of disease.
7610	.....do.......	.....do.......	Tuberculosis of mesenteric glands.
7611	.....do.......	.....do.......	Tuberculosis of liver, spleen, and abdominal lymph glands.

MILK STRAINED THROUGH CLOTH.

No. of guinea pig.	Date of injection.	Date of death.[a]	Remarks.
7614	June 19, 1906	July 24, 1906	Generalized tuberculosis.
7615	.....do.......	.....do.......	Do.
7616	.....do.......	June 25, 1906	Died, affected with peritonitis.
7617	.....do.......	June 21, 1906	Do.

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[a] Except where the word "died" is used in the remarks, the guinea pigs remained alive until they were killed for post-mortem examination.

Results of injecting guinea pigs with normal milk (from healthy cows) soiled with feces from cow No. 113 (tuberculous).[a]

MILK NOT STRAINED.

No. of guinea pig.	Date of injection.	Date of death.[b]	Remarks.
7503	May 29, 1906	July 23, 1906	Generalized tuberculosis.
7504	.....do.......	.....do.......	Tuberculosis of liver and spleen.
7505	.....do.......	.....do.......	Do.
7506	.....do.......	.....do.......	Generalized tuberculosis.
7507	.....do.......	.....do.......	Tuberculosis of liver, spleen, and abdominal lymph glands.
7508	.....do.......	.....do.......	Do.
7573	June 9, 1906	.....do.......	Generalized tuberculosis.
7574	.....do.......	.....do.......	Do.
7575	.....do.......	.....do.......	Do.
7576	.....do.......	.....do.......	Do.
7592	June 18, 1906	July 24, 1906	Do.
7593	.....do.......	.....do.......	No lesions of disease.
7594	.....do.......	.....do.......	Tuberculosis of liver.
7595	.....do.......	.....do.......	Tuberculosis of spleen.

MILK STRAINED THROUGH CLOTH.

7596	June 18, 1906	July 23, 1906	Tuberculosis of liver and spleen.
7597	.....do.......	.....do.......	Generalized tuberculosis.
7598	.....do.......	.....do.......	Tuberculosis of liver, spleen, and abdominal lymph glands.
7599	.....do.......	.....do.......	Do.

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[a] In connection with this cow it should be observed that 17 of a total of 18 guinea pigs inoculated with milk that was soiled with her feces became affected with tuberculosis, and that the amount of feces added to the milk was so small that not one guinea pig of the 18 that received intraperitoneal injections became affected with peritonitis.

[b] Except where the word "died" is used in the remarks, the guinea pigs remained alive until they were killed for post-mortem examination.

Results of injecting guinea pigs with normal milk (from healthy cows) soiled with feces from cow No. 84 (tuberculous).

MILK NOT STRAINED.

No. of guinea pig.	Date of injection.	Date of death.[a]	Remarks.
7638	June 21, 1906	July 25, 1906	Tuberculosis of lung, liver, and spleen.
7639	.....do.......	July 5, 1906	Died, affected with peritonitis.
7640	.....do.......	July 25, 1906	Tuberculosis of liver and spleen.
7641	.....do.......	June 26, 1906	Died, affected with peritonitis.
7642	.....do.......	July 25, 1906	Tuberculosis of liver and spleen.

MILK STRAINED THROUGH CLOTH.

7643	June 21, 1906	July 25, 1906	Tuberculosis of liver and spleen.
7644	.....do.......	June 22, 1906	Died, affected with peritonitis.
7645	.....do.......	.....do.......	Do.
7646	.....do.......	.....do.......	Do.
7647	.....do.......	.....do.......	Do.
7673	June 22, 1906	July 24, 1906	No lesions of disease.
7674	.....do.......	.....do.......	Do.
7675	.....do.......	.....do.......	Do.
7676	.....do.......	.....do.......	Do.
7677	.....do.......	.....do.......	Do.
7678	.....do.......	.....do.......	Do.

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[a] Except where the word "died" is used in the remarks, the guinea pigs remained alive until they were killed for post-mortem examination.

Results of injecting guinea pigs with normal milk (from healthy cows) soiled with feces from cow No. 373 (tuberculous).

MILK NOT STRAINED.

No. of guinea pig.	Date of injection.	Date of death.[a]	Remarks.
7624	June 20, 1906	July 24, 1906	No lesions of disease.
7625	.....do.......	.....do.......	Do.
7626	.....do.......	.....do.......	Do.
7627	.....do.......	.....do.......	Do.
7549	June 7, 1906	June 9, 1906	Died, affected with peritonitis.
7550	.....do.......	June 16, 1906	Do.
7551	.....do.......	July 23, 1906	Generalized tuberculosis.
7552	.....do.......	.....do.......	Do.
7753	.....do.......	.....do.......	Do.
7554	.....do.......	.....do.......	Do.
MILK STRAINED THROUGH CLOTH.			
7630	June 20, 1906	July 24, 1906	No lesions of disease.
7631	.....do.......	.....do.......	Do.
7632	.....do.......	.....do.......	Do.
7633	.....do.......	.....do.......	Do.

As the tables show, both the strained and unstrained milk soiled with feces from cows Nos. 1, 84, and 113, and the unstrained milk soiled with feces from cow No. 373, were infectious for guinea pigs. Add to this cow No. 325 with negative results, and we have one cow that was fed tubercle bacilli in her drinking water and four affected with naturally acquired tuberculosis. The former and three of the latter were passing tubercle bacilli in their feces in sufficient numbers and of sufficient virulence to make the infection of milk exposed in their environment easily possible. No better evidence can be presented to support the conclusion that the presence of a single tuberculous cow in a dairy stable, even if her milk is not used, should not be tolerated. She is a direct danger to the healthy cattle that are exposed to her, and may be responsible for the introduction of infectious material into their milk, and thus make it dangerous for use by persons or animals.

It must not be supposed that the cows used in these tests were old, invalid animals, so badly affected with tuberculosis that no conscientious dairyman would retain them in his herd. With the single exception of cow No. 113, their condition, as far as could be determined by a simple, unaided, physical examination, was better than that of the majority of dairy cows in actual use. They looked better nourished, and their hair was smoother, glossier, and cleaner. The slight, infrequent cough with which they were affected would not have attracted the attention of the casual observer, and might have been honestly attributed by most dairymen to dust in the air of the stable. These cows illustrate anew the fact that the danger from tuberculosis does not rest on an intensely infectious character of the disease, but on its insidiousness and concealed and secret nature, which enables it to come and develop and spread infection without warning. Strictly speaking, the best as well as the worst cow in appearance relative to health must be suspected until she has been proven sound by a tuberculin test.

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[a] Except where the word "died" is used in the remarks, the guinea pigs remained alive until they were killed for post-mortem examination.

ADDITIONAL FEEDING AND INOCULATION TESTS.

Two additional tests were made for the presence of tubercle bacilli in the environment of tuberculous cattle. (1) The mixed feces of cows Nos. 1 and 113 were fed, together with cornmeal and bran, to four healthy hogs, and (2) earth from the outside of the stable in which the tuberculous cattle were housed, near the door through which the manure was removed, was microscopically examined and used for guinea-pig inoculations.

Before the feeding experiments began the hogs used in them were tested with tuberculin without showing a reaction, and hence must be regarded as originally free from tuberculosis. The feeding was continued about six weeks, and shortly after it was stopped the hogs were again tested with tuberculin. With the second tuberculin test all four gave the reaction indicative of the presence of tuberculosis. When the hogs were killed and examined post-mortem it was found that one was affected with tuberculosis of the lung and liver and the submaxillary, prescapular, and mesenteric lymph glands, and the other three with tuberculosis of the submaxillary lymph glands. The submaxillary glands, not alone from the results obtained with this experiment, but also from other observations on a larger number of animals, must be regarded as the first structures in the bodies of hogs to become affected when they contract tuberculosis through the ingestion of infectious material.

The fact that the four hogs contracted tuberculosis shows that the feces contained active, virulent tubercle bacilli, and likewise, as was pointed out in a former publication,[a] that much of the tuberculosis that occurs among hogs may be due to a method of feeding extensively practiced in the West—turning hogs behind cattle—when insufficient care is taken against the presence of tuberculosis among the cattle. Hogs that are exposed to cattle feces or have access to manure heaps from stables in which cattle are kept may at any time become tuberculous if the cattle are affected. This practice and the feeding of skim milk, especially when tubercle bacilli have been concentrated in it by the separation of the cream from the milk with a centrifugal machine, are probably the most frequent causes of tuberculosis among hogs. That tubercle bacilli may be dropped, splashed, sprayed, or switched into milk when it is obtained from tuberculous cows or has been exposed to the environment of tuberculous cattle has already been pointed out, but can not be too often repeated.

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[a] Bulletin 88, Bureau of Animal Industry.

The microscopic examination of the earth from the outside of the stable in which the tuberculous cattle used in these experiments were kept revealed the presence of numerous acid-fast bacilli. Most of the guinea pigs inoculated with specimens of this earth died from septicemia, and those that lived until they were killed for post-mortem examination showed either no lesions of disease (in a few instances) or very extensive lesions that could easily be mistaken for tuberculosis, but were shown by microscopic examination to be nontuberculous and due to the action of other bacteria than tubercle bacilli. Hence we can not assert definitely that the acid-fast bacteria found in the samples of earth on microscopic examination were tubercle bacilli, as acid-fast bacteria are very common in the soil in and around stables and barns.

CONCLUSIONS.

1. Tubercle bacilli are disseminated with the feces of tuberculous cattle. This is shown to be the case by microscopic examination, by inoculation tests with guinea pigs, and by ingestion experiments with hogs.

2. Feces are the most dangerous factor in the dissemination of tubercle bacilli by cattle affected with tuberculosis. In this respect feces must be regarded as having a place with cattle similar to that commonly accorded to sputa with tuberculous persons.

3. It is not alone the feces of visibly affected cattle which disseminate tubercle bacilli in a way that is dangerous to man and animals, but also the feces of cattle so slightly affected that the diagnosis of tuberculosis with them depends entirely on the application of the tuberculin test.

4. Tubercle bacilli that are swallowed by cattle are to a great extent passed entirely through the digestive tract and out with the feces without loss of infectiousness. As cattle do not expectorate, the infectious matter that is coughed up from their lungs is swallowed, passed through their bodies, and scattered with their feces.

5. Bacilli may reach the environment of tuberculous cattle from their mouths, but this is evidently of rare occurrence compared with the dissemination through feces, especially when the cattle are not visibly tuberculous.

6. The nasal discharge of tuberculous cattle was found to be free from infectious material. Cow No. 113 is so severely affected with

tuberculosis that this freedom in her case seems to indicate that nasal discharge rarely contains tubercle bacilli. It is possible that a larger number of tests may modify this conclusion.

7. Urine is probably free from tubercle bacilli when the genito-urinary organs are not affected and no infectious material has been introduced into it after it has been passed. The practical significance of this conclusion lies in its bearing on the question whether tubercle bacilli are ever thrown out by tuberculous subjects through unaffected secretory organs with otherwise normal secretions.

8. Milk from tuberculous cows with unaffected udders we believe to be free from infection until it has become contaminated with feces or some other material that contains tubercle bacilli from the outside of the cows or from their environment; that is to say, it is not believed that tubercle bacilli are eliminated with the milk from tuberculous cows unless disease of the udder or structures connected with it is present. This conclusion is drawn from the present series of investigations and is supported by our earlier work relative to the milk of tuberculous cows. The present investigations include only a few cows and a comparatively small number of guinea pigs. The earlier investigations extend over a dozen years, during which milk from scores of tuberculous cows was injected into the abdominal cavities of hundreds of guinea pigs.

When milk injections into guinea pigs are made by pathologists or bacteriologists as a test for the presence of infectious material, unusual, though not always sufficient, precautions are taken at the time of milking to protect the milk from contamination with foreign matter of any and every kind that may reach it from the exterior of the cow or her environment. It follows that the scientific injections give nearly accurate results as to the frequency with which tuberculous cows pass tubercle bacilli with their milk; but they give no data at all as to the frequency with which milk from tuberculous cows, or healthy cows in a tuberculous environment, contains infectious material when it is drawn and handled with the ordinary precautions that a dairyman can economically practice.

The observations made by the writers definitely show that the frequency with which milk contains tubercle bacilli is greatly underestimated, especially when it is milked in the customary way from tuberculous cows with healthy udders, or from entirely healthy cows in a tuberculous environment.

9. It has been positively shown that the introduction of a small quantity of feces from tuberculous cattle into normal milk is equivalent to the introduction of a sufficient amount of infectious material to cause a generalized tuberculosis in guinea pigs that are given intra-abdominal injections of small amounts of such soiled milk. This

is true not only with the feces of a severely affected cow, like No. 113, or a cow that is swallowing cultures of tubercle bacilli, like No. 84, but also with the feces of cows that are not known to be affected with tuberculosis until a tuberculin test or post-mortem examination has been made, as cows Nos. 1 and 373.

The quantity of feces introduced into the milk was no greater than frequently enters with ordinary milking.

10. We are unacquainted with any means by which it can be determined when cattle or their feces become dangerous to the health of persons or animals; hence every cow known to be affected with tuberculosis must be regarded as positively dangerous. Physical condition gives no information from which it is possible to determine how seriously a cow is affected with tuberculosis or how freely she is scattering tubercle bacilli. Cattle affected with advanced tuberculosis from which infection is being disseminated in a dangerous way may retain the appearance and give the general impression of perfect health. Frequently nothing abnormal can be detected about them after the most searching examination by the owner or even by a trained veterinarian; and besides it is not customary to make careful examinations or to employ professional men to do so until cattle show marked symptoms of disease.

11. In order to guard against the spread of tuberculosis among cattle and other animals, and more especially for the protection of persons, every dairy cow should be periodically tested with tuberculin, and every cow that shows a reaction indicating that she is affected with tuberculosis should at once, regardless of her general appearance or condition or semblance of health, be removed from use as a dairy cow and from all contact with dairy cattle or other healthy animals. If segregation is practiced, it should be complete, so that no healthy animal will be exposed to feces that may swarm with living, virulent tubercle bacilli.

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GENERAL [illegible]

Issued May 31, 1907.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN NO. 100.

A. D. MELVIN, CHIEF OF BUREAU.

THE GERMICIDAL VALUE OF LIQUOR CRESOLIS COMPOSITUS (U. S. P.).

BY

C. N. McBRYDE, M. D.,
Bacteriologist, Biochemic Division.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1907.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., April 12, 1907.

Sir: I have the honor to transmit herewith, and to recommend for publication as Bulletin No. 100 of this Bureau, a paper entitled "The Germicidal Value of Liquor Cresolis Compositus (U. S. P.)," by Dr. C. N. McBryde, bacteriologist in the Biochemic Division.

The substance termed "liquor cresolis compositus" has recently been introduced into the United States Pharmacopœia, and the results of Doctor McBryde's experiments show that it possesses a germicidal value which exceeds that of carbolic acid for the bacteria upon which it has been tested. It therefore seems that this solution may be of considerable importance as a disinfectant.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.

Introductory........ 7

Standard methods of testing disinfectants........ 7

The drop method........ 8

The rod method........ 8

Technique of present experiments........ 9

Comparison of carbolic acid with lime and carbolic-acid mixture........ 9

Comparison of carbolic acid with liquor cresolis compositus made from commercial cresols........ 11

Comparative value of solutions of liquor cresolis compositus made from different cresols........ 14

Separation of the three cresols from commercial cresol........ 14

Germicidal value of liquor cresolis compositus made from the three cresols. 15

Comparison of carbolic acid with liquor cresolis compositus made with low-boiling cresol........ 18

Experiments with *Bacillus tuberculosis*........ 20

Experiments with *Bacillus anthracis*........ 22

Summary of results........ 24

THE GERMICIDAL VALUE OF LIQUOR CRESOLIS COMPOSITUS (U. S. P.).

INTRODUCTORY.

The following tests were undertaken in connection with the question of disinfecting cattle cars. Heretofore the United States Department of Agriculture has recommended the use of a carbolic acid and lime mixture for the disinfection of cattle cars, pens, chutes, etc. As there were certain objections to the use of this mixture it seemed advisable to determine whether a cheaper and more efficient disinfectant could not be found for this purpose, and "liquor cresolis compositus," which has been recently added to the United States Pharmacopœia, seemed a promising substitute.

Liquor cresolis compositus is a liquid soap containing 50 per cent of cresols, which have been shown to possess high disinfectant properties. It is a thick, dark brown, oily looking liquid and is miscible with water, giving clear, saponaceous, frothing solutions.

A review of the literature on disinfectants fails to reveal any previous tests with liquor cresolis compositus (U. S. P.), and the object of the tests described in the following paper was to determine its efficiency as a disinfectant in comparison with carbolic acid, which is usually adopted as a standard of comparison in testing disinfectants.

In testing the efficiency of a disinfectant the ideal method would be to apply the tests under the same conditions that prevail in practice, according to the purpose for which the disinfectant is to be employed. In the present instance, however, where the object in view was the disinfection of cattle cars, chutes, pens, etc., this would have been quite impracticable on account of the wide variation in conditions which would be met with in practice, and it was therefore deemed best to adopt some standard method.

STANDARD METHODS OF TESTING DISINFECTANTS.

Of the various methods devised for testing disinfectants, the best known perhaps are the "thread," the "drop," the "garnet," and the "rod" methods.

In a recent publication[a] the disinfectant standardization committee of the Royal Sanitary Institute of London reports in favor of the drop method. This report is based on an extensive series of experiments conducted by Firth and Macfadyen to determine the comparative value of the thread, drop, and garnet methods. The committee considered the drop method superior to both the thread and garnet methods, not only because of its simplicity and facility, but because it yielded sharper and more definite results. The committee considered the thread method too elaborate for general use and "eminently unfitted for working with micro-organisms at all sensitive to desiccation."

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[a] Journal of the Royal Sanitary Institute, Vol. XXVII, No. 1, 1906.

As to the garnet method, the committee found that the results obtained by this method are "too irregular to justify its adoption as a means of obtaining any comparative figure of disinfecting efficiency."

In the following paper the tests were made according to the drop and rod methods, which may be briefly described as follows:

THE DROP METHOD.

This is one of the simplest and most widely used methods of testing disinfectants, and has been variously modified. The drop method employed in these experiments is that described by Rideal and Walker,[a] and is the one which was adopted by the British standardization committee, referred to above, as the most satisfactory method of testing disinfectants. The procedure in this method is to add 5 drops of a 24-hour culture of the organism upon which the disinfectant is to be tested, grown in beef broth, to 5 c. c. of the disinfectant. The mixture is then well shaken, and subcultures are made from it at intervals of 2½ minutes up to 15 minutes. These subcultures are then incubated at 38° C. for at least 2 days.

THE ROD METHOD.

This method was devised by Hill[b], and may be regarded as a convenient modification of the thread method. Glass rods three-sixteenths of an inch in diameter and 8 inches long are etched at one end with diamond ink. Cotton is then wrapped about the rods near one end, and the rods are thrust into test tubes so as to engage the cotton in the mouth of the tube. The tubes containing the rods are then sterilized by dry heat. In making the tests the rods are removed from the test tubes and the inclosed or etched portion dipped into a suspension of the organism, and then held in the air for a few moments until dry. The rods are then transferred to test tubes containing 5 c. c. each of the disinfectant to be tested and exposed to the action of the disinfectant for varying lengths of time. After exposure to the disinfectant the rods are dipped in sterile water in order to remove all traces of the disinfectant and are transferred to tubes of sterile beef broth, which are incubated for at least two days at 38° C. The suspension of the organism into which the rods are dipped is made from a 24-hour culture, grown on ordinary neutral agar, by rubbing up in several cubic centimeters of sterile water enough of the surface growth to give a suspension of approximately the same density as a 24-hour typhoid culture.

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[a] Journal of the Sanitary Institute, Vol. XXIV, 1903.

[b] Report of American Public Health Association, Vol. XXIV, 1898.

TECHNIQUE OF PRESENT EXPERIMENTS.

In carrying out the experiments the following conditions were observed: (1) All tests with disinfectants were made at room temperature, which was duly recorded for each experiment. (2) The beef broth, prepared from freshly chopped meat with the addition of peptone and salt, had a standard reaction of 1 per cent acid, as recommended by the American Public Health Association.[a] All tubes of beef broth contained 5 c.c. of the fluid. (3) All subcultures, in both the rod and drop methods, were made in beef broth, and were incubated at 38° C. for 6 days, except where growth developed earlier. (4) In the drop experiments the same platinum loop was used for inoculating the subcultures. (5) Fresh dilutions of the disinfectants were prepared for each experiment in sterile flasks with sterile distilled water.

COMPARISON OF CARBOLIC ACID WITH LIME AND CARBOLIC-ACID MIXTURE.

Before proceeding to a comparison of liquor cresolis compositus and carbolic acid it seemed advisable to compare the lime and carbolic-acid mixture recommended in the regulations of the Secretary of Agriculture[b] for the disinfection of cattle cars, pens, etc., with carbolic acid alone, in order to determine whether the addition of the lime, which was added to the carbolic acid solution merely for the purpose of showing where the latter had been applied, had any effect on the carbolic acid; that is, whether it served to increase or diminish its germicidal efficiency. A mixture of lime and carbolic acid was prepared, therefore, according to the formula given in the aforesaid regulations. This formula was as follows:

Lime	pounds..	1½
Carbolic acid	pound..	½
Water	gallon..	1

In making up this mixture for the tests which follow, chemically pure calcium oxide and crystallized carbolic acid[c] were used. The lime was weighed in a sterile flask and a sufficient quantity of the water, which had been previously sterilized by boiling, was added to insure thorough slaking. The remainder of the water was then added, and the mixture thoroughly shaken and allowed to cool. The carbolic acid crystals were then added, and the mixture was shaken well and allowed to stand for several hours to insure complete solution of the carbolic acid. After thorough shaking, portions of this mixture were diluted so as to give dilutions containing carbolic acid in the proportion of 1:100 and 1:125, and these dilutions were then tested on *Staphylococcus pyogenes aureus* and *Bacillus choleræ suis* in comparison with aqueous carbolic-acid solutions of equivalent strengths. The results of these tests, which were made according to the rod method, are shown in Tables 1 and 2.

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[a] Report of Committee on Standard Methods of Water Analysis to the Laboratory Section of the American Public Health Association, Journal of Infectious Diseases, Supplement No. 1, May, 1905.

[b] Regulations of the Secretary of Agriculture governing the inspection, disinfection, certification, treatment, handling, and method and manner of delivery and shipment of live stock which is the subject of interstate commerce. 1905. p. 9.

[c] In this and all of the following tests, crystallized carbolic acid (U. S. P.) was used.

TABLE 1.—*Comparison of carbolic acid with carbolic acid and lime.*

[Rod method. Organism tested, *Staphylococcus pyogenes aureus*, 24-hour agar culture at 38° C. Room temperature, 26° to 27° C.]

Disinfectant.	Proportion of carbolic acid in disinfectant.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Carbolic acid and lime mixture.	1:100	+	+	+	+	+	+
Carbolic acid and water........	1:100	+	+	+	–	–	–

+ means growth; – means no growth.

The subcultures were incubated for 6 days at 38° C.

TABLE 2.—*Comparison of carbolic acid with carbolic acid and lime.*

[Rod method. Organism tested, *Bacillus choleræ suis*, 24-hour agar culture at 38° C. Room temperature, 26° to 27° C.]

Disinfectant.	Proportion of carbolic acid in disinfectant.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Carbolic acid and lime mixture.	1:125	+	+	–	–	–	–
Carbolic acid and water........	1:125	–	–	–	–	–	–

+ means growth; – means no growth.

The subcultures were incubated for 6 days at 33° C.

As will be seen from Table 1, the carbolic acid and lime mixture (carbolic acid 1:100) failed to kill *S. pyogenes aureus* in 15 minutes, whereas carbolic acid alone (1:100) killed in 10 minutes. In Table 2 we see that it required an exposure of 7½ minutes to the lime and carbolic acid mixture (1:125) to kill *B. choleræ suis*, whereas an exposure of 2½ minutes to carbolic acid alone (1:125) was sufficient to accomplish the same result.

It will thus be seen that the addition of the lime to the carbolic-acid solution materially diminished its germicidal efficiency. This is no doubt due to the fact that part of the carbolic acid combines with the lime to form carbolate of calcium, which is a weaker disinfectant than carbolic acid.

COMPARISON OF CARBOLIC ACID WITH LIQUOR CRESOLIS COMPOSITUS MADE FROM COMMERCIAL CRESOLS.

The United States Pharmacopœia gives the following directions for the preparation of liquor cresolis compositus:

	Grams.
Cresol	500
Linseed oil	350
Potassium hydroxide	80
Water, a sufficient quantity to make	1,000

Dissolve the potassium hydroxide in 50 grams of water in a tared dish, add the linseed oil, and mix thoroughly. Then add the cresol and stir until a clear solution is produced, and finally sufficient water to make the finished product weigh 1,000 grams.

Two lots of liquor cresolis compositus were prepared according to the above formula from two lots of cresol, designated hereafter as Cresol I and Cresol II.

These cresols, when subjected to fractional distillation in a regulation Engel flask, gave the following results:

Cresol I.—Fifty grams of cresol were taken; first drop distilled at 185° C.; the temperature rose steadily and quite rapidly to 189° C., then slowly to 190° C., holding for some time between 190° and 191° C. The fractional distillation of this cresol gave the following result:

Below 187° C.	1.60 per cent.	This fraction was colorless.
From 187° to 189° C.	2.10 per cent.	Do.
From 189° to 195° C.	70.20 per cent.	Do.
From 195° to 200° C.	17.90 per cent.	This fraction was light straw in color.
From 200° to 203° C.	4.40 per cent.	This fraction was greenish yellow in color.
Above 203° C. (by difference).	3.80 per cent.	This fraction was dark brown in color.
	100.00 per cent.	

Cresol II.—Fifty grams of cresol were taken; first drop distilled at 185° C.; the temperature then rose quite rapidly to 195° C., then slowly to 198° C., holding for some time between 198° and 199° C. The fractional distillation of Cresol II was as follows:

Water	1.00 per cent.	
Below 187° C.	1.40 per cent.	This fraction was colorless.
From 187° to 189° C.	0.40 per cent.	Do.
From 189° to 195° C.	1.80 per cent.	Do.
From 195° to 200° C.	80.00 per cent.	Do.
From 200° to 203° C.	14.80 per cent.	This fraction was light straw in color.
Above 203° C. (by difference).	0.60 per cent.	This fraction was reddish brown in color.
	100.00 per cent.	

In preparing the liquor cresolis compositus made with Cresol II, the soapy mixture of oil and hydrate cleared up as the cresol was added, but clouded on the addition of the last few cubic centimeters of the cresol; on the addition of the water, however, the solution became clear again. In the case of the liquor cresolis compositus made with Cresol I, the mixture of oil and hydrate became clear on the addition of the cresol, but clouded on the addition of the water; after standing for 24 hours, however, the solution became clear. The liquor cresolis compositus made with Cresol II was slightly darker in color than that made with Cresol I. In making dilutions of the two lots of liquor cresolis compositus, prepared as above, it was found that the resulting solutions in both instances were turbid. A 5 per cent dilution of liquor cresolis compositus made with Cresol I gave an opaque, milky solution, from which there was no apparent separation, while a similar dilution of the liquor cresolis compositus made with Cresol II gave a brownish, turbid solution, from which there was considerable separation on standing. In view of the fact that there was some separation in the case of the liquor cresolis compositus made with Cresol II, it seemed probable that saponification was not complete, so portions of this preparation were heated for different lengths of time over a steam bath, and it was found that by heating for one hour complete saponification was effected and perfectly clear solutions could be obtained upon dilution. The two stock solutions of liquor cresolis compositus were therefore heated for one hour on the steam bath, and the following tests were made with these solutions:

TABLE 3.—*Comparison of carbolic acid with liquor cresolis compositus made from commercial cresols.*

Drop method. Organism tested, *Staphylococcus pyogenes aureus*, 24-hour broth culture at 38° C. Room temperature 26° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Carbolic acid	1:80	−	−	−	−	−	−
Do	1:100	+	+	+	+	−	−
Do	1:125	+	+	+	+	+	+
Liquor cresolis compositus (Cresol I)	1:125	+	+	+	−	−	−
Liquor cresolis compositus (Cresol II)	1:125	+	+	−	−	−	−

+ means growth; − means no growth.

The subcultures were incubated for 6 days at 38° C.

Carbolic-acid coefficient of liquor cresolis compositus made with Cresol I=1.40.

Carbolic-acid coefficient of liquor cresolis compositus made with Cresol II=1.40+.

TABLE 4.—*Comparison of carbolic acid with liquor cresolis compositus made from commercial cresols.*

[Drop method. *Bacillus pyocyaneus*, 24-hour broth culture at 38° C. Room temperature, 26° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Carbolic acid	1:125	–	–	–	–	–	–
Do	1:135	+	+	+	–	–	–
Do	1:200	+	+	+	+	+	+
Liquor cresolis compositus (Cresol I)	1:200	+	+	+	–	–	–
Liquor cresolis compositus (Cresol II)	1:200	+	+	–	–	–	–

+ means growth; – means no growth.

The subcultures were incubated for 6 days at 38° C.

Carbolic-acid coefficient of liquor cresolis compositus made with Cresol I=1.48.

Carbolic-acid coefficient of liquor cresolis compositus made with Cresol II=1.54 (average of 1.48 and 1.60).

TABLE 5.—*Comparison of carbolic acid with liquor cresolis compositus made from commercial cresols.*

Drop method. *Bacillus choleræ suis*, 24-hour beef-broth culture at 38° C. Room temperature, 26° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Carbolic acid	1:125	–	–	–	–	–	–
Do	1:135	+	+	+	–	–	–
Do	1:200	+	+	+	+	+	+
Liquor cresolis compositus (Cresol I)	1:200	+	–	–	–	–	–
Liquor cresolis compositus (Cresol II)	1:200	+	–	–	–	–	–

+ means growth; – means no growth.

The subcultures were incubated for 6 days at 38° C.

Carbolic-acid coefficient of liquor cresolis compositus made with Cresol I=1.54 (average of 1.48 and 1.60).

Carbolic-acid coefficient of liquor cresolis compositus made with Cresol II=1.54 (average of 1.48 and 1.60).

The carbolic-acid coefficients in Tables 3 to 5 are obtained as follows: A dilution of carbolic acid is first obtained which kills the organism within a given time; a dilution of the disinfectant is next obtained which kills the organism in the same time as the carbolic-acid dilution; the dilution of the disinfectant divided by the carbolic-acid dilution gives a ratio which is called the carbolic-acid coefficient. This coefficient represents the efficiency of the disinfectant expressed in multiples of carbolic acid performing the same work.

Taking an average of the carbolic-acid coefficients in the above tables we find that for the three organisms tested the two solutions of liquor cresolis compositus made from commercial cresols are approximately one and one-half times more efficient than carbolic acid. We also find that the solution of liquor cresolis compositus made with Cresol II is slightly more efficient than that made with Cresol I, which may be explained by the fact that Cresol II contains a higher percentage of the high boiling cresols, it being generally conceded by most investigators that the higher boiling cresols, i. e., paracresol and metacresol, are more efficient disinfectants than orthocresol.

In an investigation of the cresol soaps of German commerce Fehrs [a] found that cresol soaps containing the same amount of crude cresol varied in disinfectant efficiency, and he attributed this variation to differences in the chemical composition of the crude cresols from which the soaps were made; that is, to differences in the relative amounts of orthocresol, paracresol, and metacresol contained in these cresols. In the course of his investigation Fehrs found that paracresol and metacresol were both more active disinfectants than orthocresol, and he explains the varying disinfectant values of the cresol soaps on this ground.

Somewhat at variance with these results, however, are the more recent experiments of Schneider,[b] who prepared a series of cresol soaps containing equal amounts of orthocresol, paracresol, and metacresol, and found the metacresol preparation to be the most efficient, but could discover no difference in the disinfectant efficiency of the orthocresol and paracresol preparations.

In view of these somewhat conflicting results the following tests were made:

COMPARATIVE VALUE OF SOLUTIONS OF LIQUOR CRESOLIS COMPOSITUS MADE FROM DIFFERENT CRESOLS.

SEPARATION OF THE THREE CRESOLS FROM COMMERCIAL CRESOL.

Cresol II, which contained less than 1 per cent of impurities in the form of tar oils and bases, was used for this work and was purified in the following manner: Five hundred cubic centimeters of the cresol was shaken in a separatory funnel with sulphuric-acid solution (1:3) and allowed to stand until complete separation into layers had taken place. The lower (aqueous) layer, containing a sulphuric-acid extract of "organic bases," was then removed. The residue was next made alkaline with 25 per cent sodium-hydrate solution. Ether was then added, and the mixture was thoroughly agitated and allowed to stand over night to separate. The lower layer, containing the tar acids (phenols, cresols, etc.) in solution in soda as phenylates, cresylates, etc., was separated from the upper (ethereal) layer containing any tar oils present. The solution of tar acids in soda was then made acid by adding an excess of sulphuric acid (1:3) and allowed to cool; it was then agitated with ether several times until all liberated tar acids were extracted. The ether was driven off from this extract by gentle heating on a steam bath, and the residue of tar acids thus obtained was placed in a distillation flask and subjected to careful fractional distillation. The distillation was repeated three times, and three fractions approximating orthocresol, paracresol, and metacresol were collected, as follows: (1) A fraction which distilled over constantly between 187° and 189° C. (2) A fraction which came over constantly between 195° and 199° C. (3) A fraction which came over constantly between 200° and 203° C.

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[a] Centralbl. für Bakt., Parasit. und Infectionskr., Bd. XXXVII, Abt. I, 1904.

[b] Zeit. für Hyg. und Infectionskr., Bd., 53, 1906.

The boiling points of the three cresols are usually given as follows: Orthocresol, 188° C.; paracresol, 198° C.; metacresol, 201° C. There is, however, considerable variation in the boiling points of the three cresols as recorded by different observers, and because of this variation the fractions were collected within the limits stated above.

GERMICIDAL VALUE OF LIQUOR CRESOLIS COMPOSITUS MADE FROM THE THREE CRESOLS.

Solutions were prepared from each of the three fractions described in the preceding paragraph, according to the formula of the United States Pharmacopœia for liquor cresolis compositus, and the tests which follow were made with dilutions from these solutions. The stock solutions in this instance, as in the case of liquor cresolis compositus prepared from the commercial cresols, were heated for one hour on a steam bath, in order to insure complete saponification.

In the following tests a comparison was made of the drop and rod methods in addition to the comparison of the germicidal value of the different cresols:

TABLE 6.—*Comparison of solutions of liquor cresolis compositus made from cresols which boil at temperatures approximating orthocresol, paracresol, and metacresol.*

[Drop method. Organism tested, *Bacillus typhosus*, 24-hour beef-broth culture at 38° C. Room temperature, 30° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus containing—							
Cresol 187° to 189° C.	1:350	+	+	+	+	+	+
Cresol 195° to 199° C.	1:350	+	+	+	+	−	−
Cresol 200° to 203° C.	1:350	+	−	−	−	−	−

+ means growth; − means no growth.

The subcultures were incubated for 6 days at 38° C.

TABLE 7.—*Comparison of solutions of liquor cresolis compositus made from cresols which boil at temperatures approximating orthocresol, paracresol, and metacresol.*

[Rod method. Organism tested, *Bacillus typhosus*, 24-hour agar culture at 38° C. Room temperature, 28° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus containing—							
Cresol 187° to 189° C.	1:450	+	+	+	+	+	+
Cresol 195° to 199° C.	1:450	+	+	+	−	−	−
Cresol 200° to 203° C.	1:450	+	−	−	−	−	−

+ means growth; − means no growth.

The subcultures were incubated for 6 days at 38° C.

TABLE 8.—*Comparison of solutions of liquor cresolis compositus made from cresols which boil at temperatures approximating orthocresol, paracresol, and metacresol.*

[Drop method. Organism tested, *Staphylococcus pyogenes aureus*, 24-hour beef-broth culture at 38° C Room temperature, 29° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus containing—							
Cresol 187° to 189° C........	1:250	+	+	+	+	+	+
Cresol 195° to 199° C........	1:250	+	+	–	–	–	–
Cresol 200° to 203° C........	1:250	+	–	–	–	–	–

+ means growth; – means no growth.
The subcultures were incubated for 6 days at 38° C.

TABLE 9.—*Comparison of solutions of liquor cresolis compositus made from cresols which boil at temperatures approximating orthocresol, paracresol, and metacresol.*

[Rod method. Organism tested, *Staphylococcus pyogenes aureus*, 24-hour agar culture at 38° C. Room temperature, 33° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus containing—							
Cresol 187° to 189° C........	1:300	+	+	+	+	+	+
Cresol 195° to 199° C........	1:300	+	–	–	–	–	–
Cresol 200° to 203° C........	1:300	–	–	–	–	–	–

+ means growth; – means no growth.
The subcultures were incubated for 6 days at 38° C.

TABLE 10.—*Comparison of solutions of liquor cresolis compositus made from cresols which boil at temperatures approximating orthocresol, paracresol, and metacresol.*

[Drop method. Organism tested, *Bacillus coli communis*, 24-hour beef-broth culture at 38° C. Room temperature, 28° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus containing—							
Cresol 187° to 189° C........	1:250	+	+	+	+	+	+
Cresol 195° to 199° C........	1:250	+	+	+	+	–	–
Cresol 200° to 203° C........	1:250	+	+	–	–	–	–

+ means growth; – means no growth.
The subcultures were incubated for 6 days at 38° C.

TABLE 11.—*Comparison of solutions of liquor cresolis compositus made from cresols which boil at temperatures approximating orthocresol, paracresol, and metacresol.*

[Rod method. Organism tested, *Bacillus coli communis*, 24-hour agar culture at 38° C. Room temperature, 31° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus containing—							
Cresol 187° to 189° C........	1:300	+	+	+	+	+	+
Cresol 195° to 199° C........	1:300	+	+	–	–	–	–
Cresol 200° to 203° C........	1:300	+	–	–	–	–	–

+ means growth; – means no growth.
The subcultures were incubated for 6 days at 38° C.

TABLE 12.—*Comparison of solutions of liquor cresolis compositus made from cresols which boil at temperatures approximating orthocresol, paracresol, and metacresol.*

[Drop method. Organism tested, *Bacillus pyocyaneus*, 24-hour beef-broth culture at 38° C. Room temperature, 31° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus containing—							
Cresol, 187° to 189° C.	1:300	+	+	+	+	+	+
Cresol, 195° to 199° C.	1:300	+	+	–	–	–	–
Cresol, 200° to 203° C.	1:300	+	–	–	–	–	–

+ means growth; – means no growth.

The subcultures were incubated for 6 days at 38° C.

TABLE 13.—*Comparison of solutions of liquor cresolis compositus made from cresols which boil at temperatures approximating orthocresol, paracresol, and metacresol.*

[Rod method. Organism tested, *Bacillus pyocyaneus*, 24-hour agar culture at 38° C. Room temperature, 29° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresol compositus containing—							
Cresol, 187° to 189° C.	1:300	+	+	+	+	+	+
Cresol, 195° to 199° C.	1:300	+	+	+	–	–	–
Cresol, 200° to 203° C.	1:300	+	+	–	–	–	–

+ means growth; – means no growth.

The subcultures were incubated for 6 days at 38° C.

From Tables 6 to 13 it will be seen that the germicidal efficiency of liquor cresolis compositus increases with the boiling point of the cresol it contains. It will also be noted that the two solutions of liquor cresolis compositus made from the two higher-boiling cresols (i. e., those approximating paracresol and metacresol) are both very much more efficient and stand apart, as it were, from the solution made from the lowest-boiling cresol, which approximates orthocresol.

In comparing the results obtained by the drop and rod methods we find that as a rule the organisms tested were more easily killed on the rods. By the rod method *Bacillus typhosus*, *B. coli communis*, and *Staphylococcus pyogenes aureus* were killed by more dilute solutions than were required by the drop method, and it would seem probable that the resistance of these organisms was somewhat lowered by the drying to which they were subjected in the rod method. *B. pyocyaneus* was killed by the same strength of solutions in practically the same length of time with both methods, and it would seem that this organism was probably more resistant to desiccation than the ones mentioned above. From these results it would seem that the same objection that Firth and MacFadyen found to the thread method would hold for the rod method—i. e., that it is not well fitted for working with organisms that are sensitive to desiccation.

COMPARISON OF CARBOLIC ACID WITH LIQUOR CRESOLIS COMPOSITUS MADE WITH LOW-BOILING CRESOL.

In the preceding section it was found that liquor cresolis compositus made with a cresol which boils at a temperature approximating orthocresol (i. e., 187° to 189° C.) was much the weakest disinfectant of three preparations made from a series of cresols approximating orthocresol, paracresol, and metacresol. In the following tables this solution of liquor cresolis compositus, which was made from the lowest-boiling cresol of the series and found to be the weakest of the three preparations in germicidal value, is compared with carbolic acid:

TABLE 14.—*Comparison of carbolic acid with liquor cresolis compositus (cresol 187° to 189° C.).*

[Drop method. Organism tested, *Bacillus coli communis*, 24-hour beef-broth culture at 38° C. Room temperature, 25° to 28° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus (cresol 187° to 189° C.)	1:175	+	+	−	−	−	−
Carbolic acid	1:175	+	+	+	+	+	+
Do	1:125	+	+	+	+	+	+
Do	1:100	+	−	−	−	−	−

+ means growth; − means no growth.

The subcultures were incubated for 6 days at 38° C.
Carbolic-acid coefficient = 1.57 (average of 1.40 and 1.75).

TABLE 15.—*Comparison of carbolic acid with liquor cresolis compositus (cresol 187° to 189° C.).*

[Drop method. Organism tested, *Bacillus typhosus*, 24-hour beef-broth culture. Room temperature, 27° to 30° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus (cresol 187° to 189° C.)	1:200	+	+	−	−	−	−
Carbolic acid	1:200	+	+	+	+	+	+
Do	1:135	+	+	+	+	−	−
Do	1:125	+	−	−	−	−	−

+ means growth; − means no growth.

The subcultures were incubated for 6 days at 38° C.
Carbolic-acid coefficient = 1.54 (average of 1.48 and 1.60).

TABLE 16.—*Comparison of carbolic acid with liquor cresolis compositus (cresol 187° to 189° C.).*

[Drop method. Organism tested, *Bacillus choleræ suis*, 24-hour beef-broth culture at 38° C. Room temperature, 26° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus (cresol 187° to 189° C.)	1:175	+	+	−	−	−	−
Carbolic acid	1:175	+	+	+	+	+	+
Do	1:135	+	+	+	−	−	−
Do	1:125	−	−	−	−	−	−

+ means growth; − means no growth.

The subcultures were incubated for 6 days at 38° C.

Carbolic-acid coefficient = 1.35 (average of 1.30 and 1.40).

TABLE 17.—*Comparison of carbolic acid with liquor cresolis compositus (cresol 187° to 189° C.).*

[Drop method. Organism tested, *Bacillus pyocyaneus*, 24-hour beef-broth culture at 38° C. Room temperature, 27° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus (cresol 187° to 189° C.)	1:175	+	+	+	+	−	−
Carbolic acid	1:175	+	+	+	+	+	+
Do	1:150	+	+	+	+	+	+
Do	1:135	+	+	+	−	−	−

+ means growth; − means no growth.

The subcultures were incubated for 6 days at 38° C.

Carbolic-acid coefficient = 1.23 (average of 1.30 and 1.16).

TABLE 18.—*Comparison of carbolic acid with liquor cresolis compositus (cresol 187° to 189° C.).*

[Drop method. Organism tested, *Staphylococcus pyogenes aureus*, 24-hour beef-broth culture. Room temperature, 27° to 30° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		2½ minutes.	5 minutes.	7½ minutes.	10 minutes.	12½ minutes.	15 minutes.
Liquor cresolis compositus (cresol 187° to 189° C.)	1: 150	+	−	−	−	−	−
Carbolic acid	1: 150	+	+	+	+	+	+
Do	1: 100	+	+	+	+	−	−
Do	1: 80	−	−	−	−	−	−

+ means growth; − means no growth.

The subcultures were incubated for 6 days at 38° C.

Carbolic-acid coefficient=1.63 (average of 1.50 and 1.87).

Taking an average of the carbolic-acid coefficients in Tables 14–18, it will be found that for the five organisms tested the germicidal efficiency of liquor cresolis compositus made with cresol which boils between 187° and 189° C. is nearly one and one-half times greater than that of carbolic acid.

We thus see that a solution of liquor cresolis compositus made from the lowest-boiling and least effective cresol (i. e., cresol 187° to 189° C.) has a higher disinfectant value than carbolic acid, and we may safely conclude, therefore, that a solution of liquor cresolis compositus made from any cresol which boils at the temperatures specified by the United States Pharmacopœia (195° to 205° C.) would surpass carbolic acid in disinfectant efficiency.

EXPERIMENTS WITH BACILLUS TUBERCULOSIS.

A culture of *Bacillus tuberculosis* grown at 38° C. on beef broth containing peptone, glycerin, and acid potassium phosphate was selected for this experiment. The culture was 17 days old, and showed a well-developed pellicle covering the entire surface. Several bits of the growth were removed by means of a platinum loop, placed in the bottom of a dry, sterile test tube, and thoroughly macerated by means of a heavy platinum rod flattened at the end. Three cubic centimeters of normal salt solution was then added, and some bits of sterile glass, and the tube was thoroughly agitated. The tube was then allowed to stand for about half an hour, at the end of which time the coarser particles had settled out, leaving a homogeneous suspension of about the density of a 48-hour typhoid culture. This suspension was carefully drawn off by means of a sterile pipette, and transferred to a dry, sterile test tube. Sterile glass rods with etched ends (Hill's method) were then dipped in this suspension, and allowed to dry for a few moments in the air. The rods were then exposed for varying intervals of time to 1 per cent solutions of carbolic acid and liquor cresolis compositus (cresol 187° to 189° C.). After exposure to the antiseptic each rod was dipped in a large test tube containing 50 c. c. of sterile water in order to get rid of all traces of the disinfectant and the bacilli were then carefully washed from the rods in 2 c. c. of sterile salt solution in a small sterile beaker. A small glass rod, tipped with rubber tubing and sterilized by boiling, was used in washing the tubercle bacilli from the end of the etched rod, the latter being thoroughly rubbed in the salt solution with the rubber-tipped rod, so as to remove all of the tubercle bacilli adhering to the etched portion. The salt solution containing the tubercle bacilli which had been exposed to the action of the disinfectant was then injected into a guinea pig beneath the skin of the flank. Guinea pigs were also inoculated from check rods which had received a film of cilli and had then been dipped in sterile water and washed off in salt solution in the same manner as those which were exposed to the disinfectant. The room temperature at the time the rods were exposed to the action of the disinfectant was 28° C. The guinea pigs used for these experiments were of uniform size and weight, the average weight being about 375 grams.

TABLE 19.—*Showing effect of 1 per cent carbolic acid on Bacillus tuberculosis.*

[Rod method. 17-day beef-broth culture No. 128 at 38° C.]

No. of guinea pig.	Material injected.	Date of inoculation.	Days alive after inoculation.	Result.
7708	Culture not exposed to disinfectant	July 16, 1906	35	Died. Tuberculous.
7711	do	do	51	Do.
7709	Culture exposed to disinfectant for ½ minute	do	12	Died. Pneumonia.
7707	Culture exposed to disinfectant for 1 minute	do	83	Died. Tuberculous.
7710	Culture exposed to disinfectant for 2 minutes	do	91	Died. Nontuberculous.
7584	Culture exposed to disinfectant for 5 minutes	do	91	Do.

The autopsy on guinea pig 7709, which died within 12 days after inoculation, showed consolidation of the anterior lobes of both lungs and this animal probably died from an intercurrent pneumonia. Had the animal lived it would in all probability have developed tuberculosis, as the animal next above it in the series—guinea pig 7707—inoculated with tubercle bacilli which were given a longer exposure to carbolic acid, developed tuberculosis and died with well-marked lesions.

It will be noticed that guinea pig 7707 lived for 83 days after inoculation, whereas the two checks—guinea pigs 1708 and 1711—died within 35 and 51 days of the inoculation. Taking an average for the two checks it will be seen that guinea pig 7707 lived nearly twice as long as did the check animals and it would seem from this that the tubercle bacilli with which guinea pig 7707 was inoculated had been considerably attenuated by an exposure of 1 minute to carbolic acid, or else that a large number of the bacilli were killed, thus reducing the dose of live bacilli which the animal received and consequently prolonging the course of the disease.

At the time guinea pigs 7710 and 7584 died a number of guinea pigs were dying from unknown causes, but careful autopsies on these animals failed to reveal any traces of tuberculosis and it would seem that the tubercle bacilli with which these animals were inoculated had been completely killed by the carbolic acid.

TABLE 20.—*Showing effect of 1 per cent liquor cresolis compositus (cresol 187° to 189° C.) on Bacillus tuberculosis.*

[Rod method. 17-day beef-broth culture No. 128 at 38° C.]

No. of guinea pig.	Material injected.	Date of inoculation.	Days alive after inoculation.	Result.
7525	Culture not exposed to disinfectant	June 6, 1906	41	Killed. Tuberculous.
7517	do	do	41	Do.
7520	Culture exposed to disinfectant for ½ minute	do	41	Killed. Nontuberculous.
7516	do	do	41	Do.
7523	Culture exposed to disinfectant for 1 minute	do	42	Do.
7515	do	do	42	Do.
7524	Culture exposed to disinfectant for 2 minutes	do	42	Do.
7514	do	do	42	Do.
7522	Culture exposed to disinfectant for 5 minutes	do	32	Died. Nontuberculous.
7513	do	do	40	Do.

In the experiment recorded in Table 20 the two check animals—guinea pigs 7525 and 7517—both showed well-marked lesions of tuberculosis at the end of 6 weeks, whereas none of the animals inoculated with tubercle bacilli which had been exposed to liquor cresolis compositus (cresol 187° to 189° C.) showed any trace of tuberculosis.

Comparing Tables 19 and 20, we find that it required an exposure of two minutes to 1 per cent carbolic acid to kill *B. tuberculosis*, whereas an exposure of one-half minute to 1 per cent liquor cresolis compositus was sufficient to accomplish the same result. In other words, liquor cresolis compositus (cresol 187° to 189° C.) killed *B. tuberculosis* in about one-fourth the time required by the same strength of carbolic-acid solution.

EXPERIMENTS WITH BACILLUS ANTHRACIS.

In all of the preceding tests purely vegetative—i. e., nonspore-bearing—micro-organisms were used, and it was shown that liquor cresolis compositus is strongly germicidal for these organisms, viz, *Bacillus pyocyaneus*, *B. choleræ suis*, *B. coli communis*, *B. typhosus*, *B. tuberculosis*, and *Staphylococcus pyogenes aureus*.

The spore-bearing micro-organisms, by reason of their spores, possess a much higher degree of resistance to both chemical and physical agents than the purely vegetative micro-organisms, and for this reason it seemed advisable to determine the germicidal efficiency of liquor cresolis compositus for such an organism. The following tests, therefore, were made with *Bacillus anthracis*, the spores of which are highly resistant to most chemical disinfectants.

The cultures used in these tests were examined microscopically before the tests were made, and showed an abundance of spores.

TABLE 21.—*Showing effect of liquor cresolis compositus on Bacillus anthracis.*

[Rod method. 10-day agar culture at 38° C. Room temperature, 28° to 30° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		5 minutes.	30 minutes.	1 hour.	3 hours.	6 hours.	24 hours.
Liquor cresolis compositus containing—							
Cresol I	5 per cent	+	+	+	+	+	+
Do	6 per cent	+	+	+	+	+	−
Do	8 per cent	+	+	−	−	−	−
Cresol II	5 per cent	+	+	+	+	+	+
Do	6 per cent	+	+	+	+	+	−
Do	8 per cent	+	+	−	−	−	−

+ means growth; − means no growth.

The subcultures were incubated for 10 days at 38° C..

TABLE 22.—*Showing effect of liquor cresolis compositus on Bacillus anthracis.*

[Rod method. 10-day agar culture at 38° C. Room temperature, 25° C.]

Disinfectant.	Strength of solution.	Result of exposure to disinfectant for—					
		5 minutes.	30 minutes.	1 hour.	3 hours.	6 hours.	24 hours.
Liquor cresolis compositus containing—							
Cresol 187° to 189° C	6 per cent	+	+	+	+	+	−
Cresol 195° to 199° C	do	+	+	+	+	+	−
Cresol 200° to 203° C	do	+	+	+	+	+	−

+ means growth; − means no growth.

The subcultures were incubated for 10 days at 38° C.

From Tables 21 and 22 it will be seen that for the destruction of anthrax spores much stronger solutions of liquor cresolis compositus would be required than for the purely vegetative micro-organisms.

In these tests, owing to the well-known resistance of anthrax spores, the lengths of exposure to the disinfectant were made longer than in the case of the purely vegetative micro-organisms, and the results, therefore, were not so sharp and definite as with the latter. Thus, in Table 21, we fail to see any difference in the germicidal efficiency of solutions of liquor cresolis compositus made from Cresol I and Cresol II, whereas in the case of the vegetative micro-organisms liquor cresolis compositus made from Cresol II was found to be the more efficient. Also, in Table 22 we fail to note any difference in the germicidal efficiency of solutions of liquor cresolis compositus made from cresols of different boiling points, whereas these solutions showed marked differences in germicidal efficiency when tested on the vegetative micro-organisms. With stronger solutions and shorter lengths of exposure, differences in the germicidal efficiency of the various solutions of liquor cresolis compositus tested would no doubt have been noted for the anthrax spores as well as in the case of the vegetative micro-organisms.

As the carbolic-acid coefficient of liquor cresolis compositus for anthrax spores was not determined, we can not make a comparison in this instance between liquor cresolis compositus and carbolic acid.

SUMMARY OF RESULTS.

(1) Liquor cresolis compositus is strongly germicidal for the following micro-organisms: *Bacillus pyocyaneus, B. choleræ suis, B. coli communis, B. typhosus, B. tuberculosis,* and *Staphylococcus pyogenes aureus.*

(2) The germicidal efficiency of liquor cresolis compositus increases with the boiling point of the cresol it contains, and solutions of liquor cresolis compositus made from the higher-boiling cresols—i. e., cresols which approximate paracresol and metacresol—are both much stronger germicides than liquor cresolis compositus made from a low-boiling cresol, which approximates orthocresol.

(3) Liquor cresolis compositus made from commercial cresols may be expected to vary somewhat in germicidal efficiency, owing to variations in the boiling points of the cresols it contains. This variation, however, does not render it unreliable as a disinfectant, as a solution of liquor cresolis compositus made from the lowest-boiling and least-active cresol was found to possess stronger germicidal properties than carbolic acid.

(4) The germicidal value of liquor cresolis compositus made from cresol which boils at a temperature approximating orthocresol (187° to 189° C.) is nearly one and one-half times greater than carbolic acid.

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